(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 September 2001 (20.09.2001)

PCT

(10) International Publication Number
WO 01/68259 A1

(51) International Patent Classification[7]: B01L 3/02, G01N 21/00, 31/00, 33/00, B32B 27/04, 27/12, 5/02

(21) International Application Number: PCT/US01/08386

(22) International Filing Date: 15 March 2001 (15.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/189,815 16 March 2000 (16.03.2000) US

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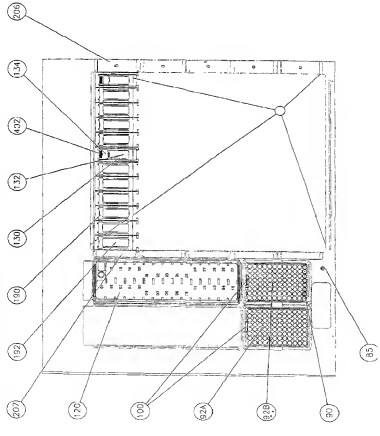
(81) Designated States *(national)*: JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: AUTOMATED SPECIMEN PROCESSING APPARATUS WITH FLUID DETECTION



(57) Abstract: An automated specimen processing apparatus (10) for dispensing a reagent onto a mounted specimen comprising a support framework (20), at least one reagent vial holder (120) movable arm (30), multifunction Z head (70). The device also includes a dispensing means that comprises at least one reagent tip head (90), first control means for positioning the tip head, means for withdrawing fluid from the vial, and a second control means to detect the fluid level in the reagent vial.

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Description

Automated Specimen Processing Apparatus with Fluid Detection

Technical Field

The present invention relates to automated equipment used in the processing of cell and tissue specimens for staining, assaying and evaluation.

Background of the Invention

Microscopic examination of unstained cell and tissue specimens often suffers from a lack of contrast between individual cells and the background matrix or between individual parts of cells. In order to alleviate this difficulty, stains that are taken up differentially by cells or parts of cells have been used for over a century.

Because of the manner in which mounted tissue samples are prepared (see Elias, J., "Immunohistopathology: A practical Approach to Diagnosis" ASCO Press, 1990, pp. 3-4, for examples of such preparation), the size and/or location of a tissue sample on a microscope slide can vary considerably within a relatively large area of the slide. In order to apply a stain to the correct location on a slide and to provide rinsing and other manipulation steps at appropriate times and in proper amounts, until recently all such staining operations were carried out by hand. However, modern laboratories that examine large numbers of tissue specimens find it desirable to automate the staining process. Accordingly, a number of manufacturers have developed equipment for automated staining of tissue samples on slides.

For example, U.S. Patent No. 4,985,206 describes an apparatus and process for automating the application of staining reagents to a thin tissue section mounted on a microscope slide. The apparatus and method use a channel-defining element that is assembled with the microscope slide to provide an enclosure of capillary dimensions into which liquids can be injected. Liquids are added sequentially to the capillary space, where the addition of a new liquid forces out the previous liquid. A plurality of these assemblies of microscope slides and specialized covers can be placed in a rack on an apparatus for automated addition of liquids.

A further automated immunostaining apparatus, known as the Ventana 320™ is produced by Ventana Medical Systems, Inc. This apparatus applies a liquid known as

Liquid Coverslip™ to each slide prior to reagent addition. Liquid Coverslip™ is a non-aqueous material having a density less than that of water. When a reagent dissolved in water is added to a microscope slide, the reagent sinks to the bottom of the Liquid Coverslip™ layer, spreading across the surface of the slide. Slides are organized on a carousel, which rotates beneath a dispensing head of the apparatus for application of reagents or wash fluids.

Yet another apparatus, known as the Jung Histostainer Ig™ Automated Immunostainer, is produced by Leica Instrument GmbH. This is also a carousel-type device, but reagents are applied by a spraying operation rather than by dropping liquid onto an organic film. The apparatus contains a permanent reagent spraying head that can be moved along a single axis to provide spray coverage over a microscope slide located on the rotating tray when the slide is rotated into position underneath the head. Excess reagent is removed by a permanent clearing nozzle, which blows air in a pressure front across the slide, forcing excess liquid off at the completion of the reagent incubation step.

A further apparatus is the subject of U.S. Patent No. 5,439,649. This device includes an arm moveable in three dimensions attached to a framework. A hollow tip head is carried on the arm, and includes a wash/blow head for dispensing reagents and clearing the slides. The reagent application tip can be attached to the hollow tip head or removed by a pre-selected movement of the arm.

In view of the ongoing need for improvement in the design and manufacture of automated specimen processing apparatus, maximizing the efficiency of the automated operations, minimizing the requirement for skilled labor in sample preparation and performance of the analytic protocols, conserving valuable reagents, as well as performing a number of separate staining protocols, often involving distinct chemistries, it is consider desirable to provide an improved apparatus which simplifies the manufacturing requirements, reduces the requirement for operator intervention during normal operation, while providing greater flexibility in operation and utilizing a wider range of simultaneous processing protocols.

Disclosure of the Invention

The present invention provides an automated specimen processing apparatus used in the processing of mounted cell and tissue specimens for staining, assaying and evaluation. The apparatus has the ability to implement a wide range of processing protocols utilizing numerous distinct reagents in processing a single batch of mounted specimens, while minimizing waste of reagents and risk of cross-contamination. In addition, the apparatus enables the use of a simplified motor arrangement in order to reduce the reliance on costly and potentially problematic components. The present apparatus also simplifies the means of calibrating the various components and detecting the locations of the mounted specimens, reagents and pipette tips by providing a common reference point for the calibration of the apparatus. Furthermore, the apparatus includes the ability to detect the fluid levels in fluid reservoirs such as reagent vials and the like, and allow the processing protocols to be implemented or revised based upon the information so detected.

In one aspect, the apparatus comprises a supporting framework including at least one mounted specimen, a reagent vial holder located on the framework and adapted for holding at least one reagent vial, and a multifunction Z head comprising reagent dispensing means attached to the framework. The reagent dispensing means comprises a reagent tip head, a first control means to direct the reagent tip head to a position from which the tip head can access the contents of the reagent vial, and means for withdrawing fluid from the reagent vial and dispensing fluid onto the mounted specimen. The apparatus further comprises a second control means operatively connected to the reagent dispensing means and adapted to cause the reagent tip head to detect the fluid level of the reagent contained in the reagent vial, and to perform at least one predetermined function based upon the detected fluid level.

Other aspects of the present invention will be readily apparent from the following more detailed description.

Brief Description of the Drawings

The invention will be better understood by reference to the following detailed description of specific embodiments when considered in combination with the drawings that form a part of this specification, wherein:

Figure 1 depicts one embodiment of the present apparatus, in which panel A is a top plan view of the apparatus with a cover, panel B is a rear elevation of the apparatus with a cover, but with portions broken away, panel C is a left horizontal elevation of the apparatus with a cover, with detail shown in phantom line, panel D is front elevation of the apparatus without a cover and panel E is a right horizontal elevation of the apparatus without a cover;

Figure 2 depicts detail of the apparatus embodiment of Figure 1, in which panel A is a top plan view of the framework of the apparatus, panel B is a front elevation of the framework, and panel C is a right horizontal elevation of the framework;

Figure 3 depicts a detail of the movable arm embodiment of Figure 1, in which panel A is a top plan view of the arm of the apparatus, panel B is a front elevation of the arm, and panel C is a right horizontal elevation of the arm;

Figure 4 depicts a detail of the Z head embodiment of Figure 1, in which panel A is a front elevation of the framework, and panel B is a left horizontal elevation of the framework;

Figure 5 depicts multiple views of the wash and blow head embodiment of the apparatus of Figure 1;

Figure 6 depicts multiple views of the wash and blow head assembly embodiment of the apparatus of Figure 1;

Figure 7 depicts multiple views of the tubing assembly of the wash and blow head embodiment of the apparatus of Figure 5;

Figure 8 depicts multiple views of the tubing distributor sub-assembly of the wash and blow head embodiment of the apparatus of Figure 5;

Figure 9 depicts tubing valve sub-assembly of the wash and blow head embodiment of the apparatus of Figure 1;

Figure 10 depicts a series of fluid reservoirs associated with the tubing valve sub-assembly of the apparatus of Figure 1;

Figure 11 depicts the dispensing head sub-assembly of the apparatus of Figure 1;

Figure 12 depicts the fluid level detecting sub-assembly of the apparatus of Figure 1;

Figure 13 depicts a system control block diagram of the apparatus of Figure 1;

Figure 14 depicts a block diagram of the electrical system of the apparatus of Figure 1;

Figure 15 depicts the Z motor sub-assembly of the apparatus of Figure 1;

Figure 16 depicts the y-axis motor sub-assembly of the apparatus of Figure 1;

Figure 17 depicts a bar-code scanner sub-assembly of the apparatus of Figure 1;

Figure 18 depicts a top plan view of the one-level work base platform of the framework of the apparatus of Figure 1;

Figure 19 depicts a top plan view of a set of retaining clip springs for the pipette rack of the apparatus of Figure 1;

Figure 20 depicts a reagent vial holder of the apparatus of Figure 1;

Figure 21 depicts a perspective view of the framework incorporating the one-level work base platform of Figure 18 and the movable arm of Figure 3 of the apparatus of Figure 1;

Figure 22 depicts a reagent tip head of the apparatus of Figure 1;

Figure 23 depicts a representative series of embodiments of reagent dispensing patterns prepared utilizing the apparatus of Figure 1;

Figure 24 depicts a series of views of a mounted specimen substrate carrier tray of the apparatus of Figure 1;

Figure 25 depicts a top plan view and a side elevation of a mounted specimen substrate carrier tray with indicator lights of the apparatus of Figure 1; and

Figure 26 depicts a calibration tool for use with the apparatus of Figure 1.

Detailed Description of the Invention

The present invention provides an automated specimen processing apparatus used in the processing of mounted cell and tissue specimens for staining, assaying and evaluation. The apparatus has the ability to implement a wide range of processing protocols utilizing numerous distinct reagents in processing a single batch of mounted specimens, while minimizing waste of reagents and risk of cross-contamination. In addition, the apparatus enables the use of a simplified motor arrangement in order to reduce the reliance on costly and potentially problematic components. The present apparatus also simplifies the means of calibrating the various components and detecting the locations of the mounted specimens, reagents and pipette tips by providing a common reference point for the calibration of the apparatus. Furthermore, the apparatus includes the ability to detect the fluid levels in fluid reservoirs such as reagent vials and the like, and allow the processing protocols to be implemented or revised based upon the information so detected.

In one aspect, the apparatus comprises a supporting framework including at least one mounted specimen, a reagent vial holder located on the framework and adapted for holding at least one reagent vial, and a multifunction Z head comprising reagent dispensing means attached to the framework. The reagent dispensing means comprises a reagent tip head, a first control means to direct the reagent tip head to a position from which the tip head can access the contents of the reagent vial, and means for withdrawing fluid from the reagent vial and dispensing fluid onto the mounted specimen. The apparatus further comprises a second control means operatively connected to the reagent dispensing means and adapted to cause the reagent tip head to detect the fluid level of the reagent contained in the reagent vial, and to perform at least one predetermined function based upon the detected fluid level.

The apparatus of the invention provides the ability to perform a wider range of analytical techniques simultaneously or sequentially than has been available heretofore, particularly immunohistochemical staining protocols, staining with special stain protocols, fluorescence *in situ* hybridization protocols and *in situ* hybridization protocols, together with protocols for specimen preparation procedures such as de-waxing and denaturation, in processing a single batch of mounted specimens, while at the same time maximizing the efficiency of the operations, minimizing the requirement for skilled labor

in sample preparation and performance of the analytic protocols, and conserving the valuable reagents utilized in the operations.

The apparatus will first be described generally along with its operation, after which the apparatus and various component parts will be described in detail with reference to the figures that form a part of this specification.

General Features of the Apparatus

The apparatus of the invention comprises a supporting framework to which an arm movable in three dimensions is attached. Motors or other means for moving the arm are provided under the control of a computer or other electronic control device that allows programming of movement of the arm between various work locations on or within the framework. At least one hollow tip head (as described below) is located on the arm so that liquids or gasses can be dispensed or withdrawn through the head to provide for the various work operations described below. In certain embodiments of the invention, the arm is configured so as to have either multiple, permanently attached tips with different functions or multiple disposable tips located on the arm at the same time, or some combination thereof. However, in preferred embodiments of the invention a single hollow tip head is provided having multiple channels connected to one or more separate pumps to which individual tips having different functions are attached. In a preferred embodiment, a portion of the hollow tip head is adapted to pick up disposable plastic pipette tips from the standard containers in which such tips are supplied (for example, Catalog No. 3510-R from E&K Scientific Products, Saratoga, CA). These disposable pipette tips are currently sold in a rack, which presents the base of the tip for insertion of a hand-held pipette body into the hollow tip, the tips being arranged in an array so that all individual tips in the container are accessible to the user. As will be apparent from the description below, the same or similar standard racks of pipette tips can be used in the apparatus of the present invention.

The apparatus of the invention integrates both a blow tip and a wash tip in the multi-function hollow tip head (hereafter termed the "Z head" because it is the primary component of the present apparatus which moves in the 'Z' dimension). The present Z head provides further improvements over prior Z head devices, in order to improve the

ability of the apparatus to implement a wide range of processing protocols utilizing numerous distinct reagents, while minimizing the risk of cross-contamination.

In the blow tip there will be an exit slit that is usually substantially equal in length to the width of a specimen mount, typically a microscope slide. If the slit is not equal to the width of the mounting substrate with which it is intended to be used, it is preferred, though not required, that the slit be slightly wider than the substrate, as a narrower slit is less efficient in removing liquid from the substrate surface in the manner as described below. However, the practical width of the slit is limited by the desire to have a number of separate mounted specimens arranged in close proximity in the apparatus of the invention and further to avoid wasting buffer or other wash solutions that are applied to a specimen. In the blowing operation that removes excess wash/buffer solution and reagent, the exit slit on the blow tip provides a "wall" of gas, typically air, that pushes excess liquid from the surface of the substrate as the tip is passed over and parallel to the length of the substrate (described below in greater detail).

Various functions described and provided in the present apparatus employ the use of positive or negative gas pressure. The means for providing such pressures are generally well known, and often utilize devices such as vacuum pumps, gas compressors, variable volume pump, and the like, representative examples of which are readily available commercially.

The wash tip (described below in greater detail) is a further orifice located in close proximity to the linear exit slit of the blow tip, and is used to deliver diverse liquid solutions, such as buffer and wash solutions, to the specimen.

In addition, additional passageways will desirably be provided in order to dispense additional solutions and reagents, all as described below in greater detail.

The framework of the apparatus is provided with holders at predetermined locations on the framework for reagent application tips (hereafter termed pipette tips), among other removable items. Thus, programming of the arm to move to a particular predetermined location and carry out a pre-selected motion or other operation discussed herein allows the individual tips to be placed onto or released from the reagent tip head. A holder for a reagent container, more typically a plurality of reagent vials (each reagent vial containing, for example, a stain or any of various solutions associated with staining),

and a mounted specimen substrate holder are also present on a framework at other predetermined locations.

Desirably, each of the holder for reagent containers, mounted specimen substrate carrier, and reagent application tip holder will be located on a single piece framework, and provided with a common reference point, so as to simplify the calibration of the apparatus preparatory to performing a series of processing steps. Thus, standardized motions of the arm can be programmed into the control unit so that individual mounted specimen substrates at specific predetermined locations in the mounted specimen substrate carrier can be treated with reagents and/or wash fluids obtained from reagent vials or from liquids supplied through the Z head on the movable arm. In addition, the attachment steps (and optionally the detachment step) for attaching pipette tips to the Z head on the movable arm can be carried out by a pre-selected movement of the arm, much in the same manner that disposable pipette tips are now pressed onto and later removed from the end of a hand-operated pipette.

One additional component of the Z head is the dispensing head sub-assembly, as depicted in Figure 11. This sub-assembly travels on the movable arm and, incorporating a single stepping motor (as depicted in Figure 15), enables the pick-up of the pipette tips, and, in conjunction with the vacuum pump, the withdrawal and dispensing of the reagents. In addition, the vertical motion of this sub-assembly is utilized to move the wash tip and blow tip assembly, as well as in discarding the used pipette tip. Thus, a single stepper motor may be utilized to perform a number of separate functions.

One additional feature of the present invention is the ability of the apparatus to detect the level of fluid in each of the reagent vials, and to respond accordingly. For example, as the tip is being lowered into the reagent vial, preparatory to withdrawing a preselected amount of the selected reagent for dispensing onto the specimen, the tip will contact the surface of the reagent fluid, and then continue to be immersed into the reagent fluid. Previously, automated apparatus would be programmed to continue this immersion until the tip approached to bottom of the vial, to insure that the reagent would be withdrawn until the vial was empty. In the present apparatus, the tip can be immersed to a predetermined depth below the surface level of the fluid, sufficient to insure that the proper amount of fluid will be available, but without requiring that the tip be fully immersed in a full vial of fluid. Heretofore, the complete immersion of the pipette tip

into a substantially full reagent vial raised concerns that the exterior surface of the pipette tip would be coated with reagent, potentially wasting valuable reagent and risking the formation of an exterior droplet of reagent which could contaminate a specimen while the tip head was traversing to its intended and desired location for dispensing reagent. In addition, the vial can be filled initially to a greater depth than previously, as the vial need not have a substantial "dead volume" to accommodate the immersion of the tip while avoiding overflow of reagent fluid. The specific operation of the fluid level detection function will be described in greater detail.

In addition to the reagent vial holders, a plurality of additional fluid reservoirs will be provided for inclusion of various reagents, buffers and the like. In a presently preferred embodiment, at least six such reservoirs will be included for storage and dispensing of (1) a solution to remove the embedding medium from mounted specimens ("de-waxing"), (2) an immunohistochemistry buffer, (3) a special stain buffer, (4) an *in situ* hybridization buffer, (5) DI water, and (6) DEPC water. The use of these various buffers and solutions will be explained in greater detail below.

Operation of the Apparatus

The present apparatus includes the ability to perform a wide range of staining operations, assays, evaluations and the like. Primary among such capabilities is the ability to perform immunohistochemical staining, staining with special stains, and *in situ* hybridization protocols, all at the same time, all as explained in greater detail in the discussion of specific embodiments.

In a typical operation of the apparatus of the invention, multiple substrates, each generally having at least one specimen (e.g. a tissue sample) at some location on its upper surface, are placed horizontally in a carrier tray that is inserted into the apparatus at a predetermined location, usually at a location having registration pins that fit into registration holes in the tray (or similar registration means) so that the individual substrates are always located in predetermined relative positions on the framework of the apparatus. This array of mounted specimens will constitute a single batch for processing by the apparatus according to the present invention. The apparatus is programmed as appropriate for the individual specimens being treated and reagent vials are placed at their own predetermined locations in the holder of the apparatus in the same manner as the

substrates in the carrier tray described above. Likewise, pipette tips are also made available for pickup by the moveable arm. For example, a standard container of 1mL disposable pipette tips can be placed at its predetermined location in the apparatus. As stated previously, it is desirable that all such components be provided with a common reference point in order to simplify the calibration of the apparatus. Towards this end, the apparatus is designed to provide holders for pipette tip racks, at least one reagent vial rack and at least one mounted specimen substrate carrier in a one-piece working base.

Once all components are in place, the apparatus will calibrate itself by locating and identifying all of the separate components of the programmed assay protocols, and will carry out all preparation, reagent application, incubation, heating (if necessary or appropriate), and sample rinsing steps to perform the desired processing operations. In a typical operating sequence, the multifunction Z head on the movable arm moves to each of the specimens being treated in a particular cycle and begins by applying liquid from the appropriate wash buffer reservoir via a liquid supply conduit to the wash tip of the Z head. The apparatus will then use the blow tip to remove excess buffer from the specimen prior to reagent delivery. This removal is accomplished by blowing gas through the tip while the head travels along the length of the specimen substrate; a 'wall' of gas thus exits the slit and removes excess buffer from the substrate, without disrupting the mounted specimen. A small amount of buffer desirably remains on the specimen to assist in reagent distribution. The Z head with the wash tip and the blow tip is lowered in close proximity to the specimen to perform the functions of applying and removing the selected liquids by the motion of the dispensing head sub-assembly.

The dispensing head sub-assembly of the Z head on the movable arm then picks up a disposable pipette tip from the pipette tip rack that has been inserted into the pipette tip holder in the apparatus. The upper portion of the tip contacts a block attached to a flag. The motion of the flag is then detected by a sensor, indicating that the tip has been secured on the adaptor and that processing can continue. In the event that the sensor does not detect the flag, the system will repeat the operation a predetermined number of cycles until proper operation is detected, or a signal is generated indicating that operator intervention is required. In certain embodiments of the present invention, all motions of the Z head and its associated sub-assemblies, such as the dispensing head sub-assembly, will be implemented by a single stepping motor, in order to insure precision and

reproducibility. The dispensing head sub-assembly of the Z head with the pipette tip attached thereis directed to the appropriate reagent vial, and the tip is lowered into the vial until the level of the fluid surface is detected, and for a predetermined depth therein. The reagent tip head then takes up the appropriate amount of the reagent to be applied to a specimen or group of specimens from the reagent vial in the reagent container holder. In the event that insufficient reagent level is detected, the staining protocol is interrupted, and can be resumed via the programmed operations once the reagent(s) has been replenished. For efficiency, a number of specimens can be treated with a single reagent at the same time. The reagent is dispensed on the specimen in a pre-assigned pattern that operates in combination with the thin liquid film on the substrate to assure spreading of the reagent over the entire surface of the substrate to which the specimen is attached. The thin liquid film allows less reagent to be used than would be required if the film were not present to assist reagent distribution.

The disposable pipette tip is then discarded by operation of the dispensing head sub-assembly of the Z head, and the movable arm moves the Z head wash and blow tips to the substrate to apply buffer and then remove excess buffer from the next group of specimens to be processed, while the present group of specimens are being incubated with the reagent. The dispensing head sub-assembly on the movable arm then picks up the next available disposable tip from the tip rack, and the appropriate reagent is drawn into the tip and applied as before. Appropriate steps are repeated until all specimens have been treated with reagent or until a reagent incubation is complete, so that reagents may then be removed from the appropriate specimens.

Once a reagent incubation is complete, the specimens are rinsed when the movable arm moves the wash and blow tips to the specimen again, and buffer is applied to the specimen to rinse off the majority of the reagent. The blow tip then removes the excess buffer from the specimen, and the specimen is rinsed a second time with the on-line buffer, if desired. This procedure of rinsing and removing excess buffer from a specimen is repeated as desired, depending upon the individual stain or other reagent and the appropriate procedure for rinsing the reagent. The control mechanism, generally a programmable computer, keeps track of the time of the various incubations and repeats the steps above as appropriate in order to apply each appropriate reagent to each of the specimens that have been inserted into the tray.

One feature of the apparatus of the invention that allows efficient use of reagents is the method of spreading reagents described above and further described in detail below. When a standard liquid staining reagent is dropped onto a glass substrate, the reagent tends to stay in the location where placed, rather than spreading over the entire surface area of the substrate. Since the location of the tissue specimen on a substrate is variable, and may not be located in the same place from one substrate to the next, automated procedures previously required that the reagent be applied over the entire area of the substrate. While this could be accomplished by applying a relatively large amount of a dilute reagent, many staining operations do not permit the use of dilute reagents, and some stains are sufficiently expensive so that applying concentrated reagent over the entire substrate, including areas where no tissue is present, would be a major cost of operation. Accordingly, a special application system has been devised for use in the apparatus of the present invention; this system can be used generally in the manner described here for other automated equipment.

The substrate to which a stain reagent will be added is first washed with an aqueous wash solution, usually a buffer that contains one or more surfactants, which reduce the surface tension of water. However, it is not entirely satisfactory merely to flood a substrate with an aqueous solution of surfactant, since a concentrated reagent added to the substrate will then be diluted on the specimen. Accordingly, the blow tip of the invention is designed so that excess buffer can be removed from the washed substrates to produce a thin film of the aqueous solution. The height of the blow tip exit slit above the specimen, the pressure of the compressed gas being blown through the tip, and the rate of movement of the tip are selected to allow a controlled amount of buffer to remain on the specimen substrate. If too much buffer remains, the reagents will be diluted as discussed above and will not work optimally. If too little buffer remains, the buffer may evaporate prior to reagent application, and the reagents will not spread sufficiently. Specific techniques for controlling the parameters of the wash and blow tip operation to select the desired amount of buffer are described below.

In addition to the buffer and use of the wash tip and blow tip as described above, the invention also provides for dispensing of reagents on the specimen substrate in a pattern that assists spreading. A pattern is selected so that a reagent is not required to diffuse for great distances through the surface film; for example, convoluted application

patterns can be selected so that the reagent need not diffuse more than one-fourth (or some other fraction) of the width of the specimen substrate. The combination of the buffer film and the application pattern (typically dropwise or in a stream) ensures adequate coverage of the specimen regardless of the location of the tissue and allows less reagent to be used than would be required in the absence of a surface film. In a typical operation, the amount of reagent added is less than that which would be required to cover the specimen if no aqueous film were present on the substrate.

In addition to these general operations and components of the invention, the apparatus of the invention can contain additional subsystems for convenience, such as fluid reservoirs, drain pans, reagent vials and other components that are described below in more detail.

System Control and Format

Another feature of the apparatus of the invention is the system control format and method. The apparatus provides two control formats to control the staining process parameters in each staining protocol: 'Open' format and 'closed' format. The 'open' format provides great flexibility for the user of the apparatus. In this format, the system allows the user to create, change, and adjust numerous system settings, running parameters and staining protocols in the processing of individual specimens on mounting substrates to meet different requirements. Those parameters not specifically entered would default to preprogrammed parameters specific to each processing protocol.

On the other hand, in the 'closed' format, the system maximizes the process automation and very little user input is needed. It is especially useful for those users who utilize large batch quantity and similar processing procedures. A bar-code technology can be used in this format. On the upper surface of each reagent vial, there will be a label affixed at a predetermined location when it is shipped. Three bar-codes are printed in close proximity to each other on the label. The information content of the bar-code can include: 1) Name of the reagent solution; 2) Manufacture date; 3) Expiration date; 4) Serial number; 5) Reagent volume. A human-readable string is also printed on the label and is shown on the sidewall of the reagent vial. In addition, there will be a pre-printed label applied to a region on the upper surface of the specimen substrate. This label is intended for the user to include certain information. The content of this bar-code will

include the name of the protocol to be used in processing the particular specimen. A human-readable string can also be printed immediately under this bar-code as well. The height of the bar-code is generally approximately 0.25 inch, so that there will remain sufficient space for the user to write any other desired information. The coding used in this application will desirably be code128, as this code can provide advantageous information density.

Before initiating an operation, the apparatus moves a laser bar-code scanner around the bar-codes located both on the specimen substrates and the reagent vials. The digital computer will then recognize the reagents and the specimens to be treated, and their respective locations, and will calculate the required volumes of the reagents. A reagent map can also be printed by the computer for user reference. If any deficiency occurs in the reagent supply, the computer will halt the processing and request additional reagent supplies. At the same time, if any unknown protocol is detected, the computer will request that the user create a new one. The programmed instructions will also allow the feature of editing a staining protocol in the "closed" run. After this verification, the computer will control the apparatus as discussed above to automatically process the staining operations.

The invention now being generally described, the same will be described in reference to the figures, using the same reference numbers throughout to represent either identical parts that appear in different views of the same embodiment or parts in different embodiments that have identical functions.

Figure 1, panels A-E, depicts a first embodiment of the apparatus **10** of the invention in plan view from above, and various elevations. In order to make visible the movable arm and other working parts of the apparatus, Figure 1 is generally illustrated without a cover such as would normally form the upper surface of the apparatus and act in concert with retaining walls to enclose the working parts, solutions, reagents and specimen substrates (hereinafter generally exemplified by microscope slides).

In Figure 1, movable arm **30**, which will carry out numerous operations of the apparatus, is visible in its home position in the rear-left corner of the interior of framework **20** which forms the cabinet surrounding the working parts of apparatus **10** (upper-left portion of Figure 1). Framework **20** is formed from various components, such

as baseplate **22** and side plate **24**, that form the cabinet. The various locations and the corresponding parts of the apparatus or materials that are inserted into the apparatus at these locations are generally visible on baseplate **22**. At the left front of baseplate **22** is a rectangular-shaped tip disposal orifice **26**. A tip disposal bin **28**, used for holding discarded pipette tips **90** (described below), is located under the baseplate **22** and is desirably designed as a drawer so that it can be withdrawn from the front of the apparatus **10** (see Figure 2). Under the specimen substrate tray **190**, there is a drain bin **27**, which can simply be a container provided with a drain line to a waste container. These and other parts of the apparatus are generally adapted to be retained in a specific location on baseplate **22** by providing matching projections and depressions, or some other means for locating the indicated part of the apparatus on the base plate at a predetermined location. The baseplate **22** is generally provided as a single piece structure to provide flexibility and facilitate an efficient change to the layout design.

Adjacent tip disposal orifice **26** in this embodiment is the predetermined location for pipette tip holder **100** (Figure 1). In this embodiment, holder **100** is adapted to retain in position two standard pipette tip racks **92a** and **92b** each containing arrays of disposable pipette tips **90**. Ordinarily such tips are supplied in racks of 96 tips, for a total storage capability of 192 tips in all. One example of an appropriate holder **100** for pipette tips **90** (actually for tip racks **92a** and **92b**) is the region on baseplate **22** around which the base of pipette tip racks **92a** and **92b** fit snugly. In this embodiment, most of the tips are suspended below the level of the pipette tip racks **92a** and **92b** (Figure 1). This orientation provides a stable configuration for the racks. In addition, the racks may be secured, for example, by using a spring clip **101** (Figure 19). In this manner, the racks can be readily inserted in place, while prevent accidental dislodging by the action of pipette tip removal.

Adjacent pipette tip racks **92a** and **92b** is a reagent vial holder **120** (Figure 20), in this embodiment in the form of reagent vial trays, in this embodiment two trays allowing the inclusion of a total of 60 reagent vials. The reagent vial holder **120** can either be affixed to the baseplate **22** or, in the manner described above, it can be adapted to be removable from the baseplate for loading with reagent vials **110** (Figure 20) in a more convenient location. The reagent vial holder **120** is adapted to be retained by the

baseplate in a predetermined location and orientation, so that any given reagent vial **110** will always be in the same relative position on baseplate **22**.

Adjacent the reagent vial holder **120** is the specimen substrate holder, in this embodiment occupied by microscope slide trays **190** (Figures 24 and 25). Each tray **190** is retained in a predetermined location and orientation relative to baseplate **22** and the remainder of the framework **20**, so that each microscope slide **130** retained in the tray is in a predetermined location relative to the baseplate **22**. One feature of the present substrate carrier tray is the contact between the tray and the included slides. Heretofore, the tray contacted the slides in a portion of the processing area, risking a capillary or other wicking effect, and the resultant loss of fluid or cross-contamination, when fluid reagents or solutions were applied. In the present slide tray, as depicted in Figure 24, the protrusions of the tray contact the slides only in frosted, non-processing areas, thus reducing the risk of fluid loss and cross-contamination. In the embodiment described herein, five slide trays allow the inclusion of a total of 60 slides for processing.

Prior to the operation of the apparatus, the system is desirably calibrated with a common reference point **85** located on baseplate **22**. This calibration ensures that the location of the slides **130** in slide tray **190**, the location of reagent vials **110** in reagent vial holder **120**, and the location of the pipette tips **90** in pipette tip racks **92a** and **92b** are properly programmed in the system memory. This calibration operation can be performed utilizing a calibration tool **86** (Figure 26). The stem **286** of the calibration tool **86** is inserted into the reagent tip head **43**. The Z-head is lowered until point **287** of the tool **86** abuts common reference point **85** on base plate **22**. The co-ordinates of point **85** are saved in the computer memory and the calibration is complete. Alternatively, this calibration operation can be carried out in an automatic manner, for example, by embedding a light source in base plate **22** at a predefined location **85** and providing a detector in the reagent tip head **43**. The calibration will then be performed based upon the intensity of light captured by the detector.

Movable arm **30** (Figure 21) is moved to different locations over baseplate **22** by the action of various motors that operate in combination with sliding tracks to precisely position the movable arm **30** at its desired location within framework **20**, in order to carry out the operations described herein. Visible in Figure 3 (at the top of the figure) and 13 is the X-axis track **32**, in this embodiment the X-axis being the principal longer horizontal

axis of the apparatus. In the embodiment shown, a single X-axis track **32** is supported at either end on bearing shafts and brackets **34a** (left) and **34b** (right). The Y-axis is the principal shorter horizontal axis of the embodiment as shown. Stepping motors (Figures 15 and 16) are used in these embodiments under the control of the computer or other control apparatus (as described below). A portion of one motor mount **33** for the X-axis motor is visible in this Figure 13. Bearing shafts **34a** and shaft supports **34b** are also visible as part of the Y-axis track. The Z-axis in this embodiment is the orthogonal vertical axis perpendicular to the plane of Figure 1, panel A.

In a preferred working embodiment, flexible electronic leads and tubing (both gas and liquid supply conduits) would be shown in this figure leading from movable arm **30** to appropriate fluid reservoirs or electronic control equipment. These leads and conduits are not shown in Figure 1 for the sake of clarity, but are described later with respect to specific portions of the apparatus. The supply conduits are desirably sufficiently long and flexible so as to withstand the rigors of frequent use. They will originate from different pumps and desirably will be bound together. The various supply conduits pass through a flexible wire carrier **35** (Figure 2) to the left side of X-axis track **32**, then through another wire carrier **36** (Figure 3) which is at the top of the X-axis track to conduct all supply conduits to movable arm **30** and Z head **70**.

The embodiment **10** of Figure 1 is also shown in Figure 2. In this front horizontal elevation of the first embodiment of the invention, movable arm **30** is shown in its home position, to which the arm returns when not in use. The home position is desirably selected to minimize interference with other operations, such as the insertion of microscope slides or disposable pipette tips into the cabinet-like interior of framework **20**.

Figures 5 and 6 are detailed views of one embodiment of the wash tip **41** and the blow tip **42** provided in the present invention. In the figure, wash tip **41** and blow tip **42** are integrated into Z head **70**. When initiating a wash and blow operation, Z head **70** on movable arm **30** is moved upward so that Z head **70** is above any interfering part of the apparatus, then movable arm **30** is moved to an appropriate location while Z head is in the raised position. When Z head **70** reaches the appropriate location above a pre-selected microscope slide, Z head **70** is again lowered to position wash tip **41** and blow tip **42** at an appropriate height above the selected microscope slide (as shown). This positioning is accomplished when the wash and blow head assembly **88** (comprising wash tip **41** and

blow tip **42**) on Z head **70** is lowered by pivot arm **301** actuated by dispensing head sub-assembly **40**.* In a preferred embodiment, wash tip **41** and blow tip **42** are positioned at one end (e.g. the front end) of microscope slide **130** and a buffer or wash liquid, supplied through liquid supply conduit **62** (not visible in this figure), flows out from wash tip **41**. The Z head **70** is moved in a single pass to the rear of microscope slide **130**. If desired, the blow operation can then be carried out on the same slide by supplying pressurized gas through a gas supply conduit to blow tip **42**. The Z head **70** is then moved back to the front position of microscope slide **130** while keeping the gas stream from blow tip **42** in motion. However, it is also possible, and preferred in some embodiments, to move wash tip **41** and blow tip **42** to a second microscope slide for the addition of buffer, so that the buffer added to the first microscope slide can remain on the slide for a pre-selected period of time prior to removal. After the buffer has been added to a pre-selected number of slides, wash tip **41** and blow tip **42** on Z head **70** are returned to the first slide of the group and the blow operation can commence.

After the wash and blow operations, the apparatus will ordinarily select a pipette tip. The movable arm **30** will be directed to a location such that the reagent tip head **43** of dispensing head sub-assembly **40** is directly above and pointing to pipette tip **90** in pipette rack **92a** or **92b** positioned onto pipette tip holder **100**. The descending dispensing head sub-assembly **40** presses reagent tip head **43** of the dispensing head sub-assembly **40** into pipette tip **90** (here a disposable pipette tip), where the pipette tip **90** is retained on reagent tip head **43** by a press fit in the same manner in which such tips are now used on hand-operated pipettes. In order to provide a reagent tip sensing function, the upper portion of tip **90** contacts block **64**, which is attached to flag **69**. When the tip **90** is elevated, thus raising flag **69**, the flag disrupts the optical signal in sensor **169**, indicating the presence of tip **90** on reagent tip head **43**. If at the end of the pickup sequence sensor **169** does not sense flag **69**, the system can be programmed to repeat the operation a predetermined number of iterations, until a signal is received, or a message may be provided to indicate that operator intervention is required.

Dispensing head sub-assembly **40** and then Z head **70** are then raised and movable arm **30** moved to position. Then pipette tip **90** mounted on reagent tip head **43** is lowered into a reagent vial **110**, which is held in a predetermined location by reagent vial holder **120**. When the surface level of the reagent fluid is detected (as described below), the tip is

lowered a further amount as directed by the computer, and, by supplying negative pressure through pipette tip **90**, a supply of reagent is drawn into pipette tip **90** for application to microscope slides. A measured volume of reagent can be withdrawn by a precise metering pump **37**, which is driven by stepping motors to supply negative air pressure to reagent tip head **43** (i.e., withdrawing air through reagent tip head **43**). The application concept of a metering pump used herein is that a piston in the pump will withdraw a specific volume of gas and thus draw up a specific volume of reagent into pipette tip **90**. According to experience and a well-defined conversion table (volume vs. number of steps), the accuracy of the volume can be controlled as appropriate.

The fluid level detection system of the present invention is described with reference to Figure 12. In general operation, the present apparatus can detect the level of fluid in each of the reagent vials, and to respond accordingly. This feature is provided in the presently-described embodiment as a three component system: a pressure sensor **81**, a controlled static air pressure supply **83** and a computer-based data acquisition feature with analog or digital input **87**. A steady low positive pressure (e.g. 0.5 to 1psi) of compressed air will be supplied from the air supply **83** through the pipette tip **90** as the tip is being lowered into the reagent vial **110**, preparatory to withdrawing a preselected amount of the selected reagent for dispensing onto the slide. At the same time, the computer will continually but discretely sample the pressure data from the sensor **81**, with the data samples (e.g. four) being averaged to eliminate any extraneous noise and sample error. When the tip **90** contacts the surface of the reagent fluid, blocking the flow of the compressed air through the pipette tip **90**, a pressure change back-pressure is created which can be detected by the sensor **81** and interpreted by the computer. The tip **90** is then lowered further to be immersed into the reagent fluid to the desired depth sufficient to insure that the proper amount of fluid will be available, but without requiring that the tip be fully immersed in a full vial of reagent fluid. In this manner, the computer can also calculate the amount of reagent remaining in the vial, and make a determination as to the proper course of action. In the even that fluid is not detected before a predetermined tip depth is reached, the computer can interpret this result as an exhaustion of the reagent fluid supply, and the operation can be suspended accordingly. Alternatively, the level of the fluid can be detected in the reagent vials by utilizing e.g. an optical or an electrical probe integrated with reagent tip head **43**. In an optical embodiment, this function may

be provided by disruption of a light beam by the presence of the reagent that would be detected by an optical sensor. In an electrical embodiment, this function may be provided by detecting the current flow path established via the presence of the reagent.

After a calculated amount of reagent is withdrawn, Z head **70**, dispensing head sub-assembly **40**, and pipette tip **90** are then raised as before and moved to position above a pre-selected microscope slide **130**. At the pre-selected position, pipette tip **90** is lowered, and the reagent is applied to the slide. Reagent can be applied to a single microscope slide **130**, or aliquots of the reagent in pipette tip **90** can be applied to different slides according to the calculation of the selected program instructions in the computer control system.

After the final aliquot of reagent is added to the last slide, movable arm **30** is directed to a position above tip disposal orifice **26**. A tip ejection function is provided by subassembly **64**, located on dispensing head sub-assembly **40** of Z head **70** and adapted to move along the Z-axis of the apparatus. A tip ejection terminal block **63** is located at the side of dispensing head sub-assembly **40** above tip ejection subassembly **64**. When it is desired to dispose of pipette tip **90**, dispensing head sub-assembly **40** will be raised until the top end of pipette tip **90** contacts terminal block **63** installed on the Z head **70**. Since further motion of pipette tip **90** is blocked by terminal block **63**, while dispensing head sub-assembly **40** continues to rise, the lower end of tip ejection sub-assembly **64** will press against pipette tip **90** and force the tip off of the end of reagent tip head **43**. The pipette tip **90** then drops through tip disposal orifice **26**, and descending into tip disposal bin **28** (not shown) under framework **20** for later removal. The reagent tip head **43** is then lowered to a normal position so that another pipette tip **90** can be installed on reagent tip head **43**.

It should be noted that in the embodiments hereinbefore described, the location assigned to pipette tip holder **100** is clearly separate from the location assigned to reagent vial holder **120**. As an alternative embodiment, these locations could be combined, for example, in an arrangement that stores a single pipette tip **90** in association with each reagent vial **110**. In this embodiment, it is contemplated that each time a selected vial **110** is accessed, the reagent tip head **43** would be directed to the associated pipette tip **90** which would then be returned to the holder **100** for reuse after the reagent had been dispensed onto the microscope slide **130**. The control means of the apparatus **10** could

monitor the volume of reagent remaining in each reagent vial **110**, and arrange for disposal and replacement of the vial **110** and pipette tip **90** as appropriate.

One feature in this embodiment of the invention is a highly integrated, multi-functional movable arm **30**. Figure 3 depicts three views of the movable arm 30. Z head **70** and dispensing head sub-assembly **40** with reagent tip head **43** without a pipette tip **90** on the head are mounted on movable arm **30**. The Z head **70** is mounted on the Z-axis track **39** and screw lead slide **38**. Z head **70** is driven by a Z-axis motor (of which only the output gear **50** is shown in the horizontal front elevation of the figure). A laser bar-code scanner **410** (Figure 17) is located on the side of the Z head **70**. There are two scanner shields **66** installed on laser scanner **410**. The shields obscure a portion of the laser beam output window **67** and leave the sensor window **68** un-obstructed. By adjusting the shields **66** to a position so that the width of the laser beam after shielding is slightly longer than the length of the bar-code, only one bar-code is read at a time. Since the scanner sensor window **68** is unobstructed, the sensitivity is unchanged. The distance between the laser scanner **410** and the bar-code to be read must be adjusted to insure that the scanner can read the bar-code uniformly. Above the laser scanner **410**, is a metering Lee pump **37** which drives the dispensing head sub-assembly **40** and reagent tip head **43** to withdraw and dispense reagent solutions. A Z head latch can also be installed on the left side of movable arm **30**. When the apparatus **10** is shipped, the Z head latch locks to the Z-axis track **39**. The latch will release when Z head **70** returns to its home position. The gas and liquid supply conduits and connection leads are not shown in the drawing, so that the major parts can be seen more clearly. The movable arm **30** is mounted on the X-axis track **32** and can be directed linearly along the track under the direction of a computer or other means of control.

Figure 4 provides a more detailed view of the dispensing head sub-assembly **40** and reagent tip head **43** on Z head **70**. In the figure, the dispensing head sub-assembly **40** and reagent tip head **43** is depicted at the lower left side of Z head **70**. In the embodiment shown in Figure 22, reagent tip head **43** has three different diameters at different cross-sections, namely: A segment **43a** that is sufficiently large to act as a stop when press-fitting pipette tip **90** onto reagent tip head **43**; an intermediate section **43b** that acts as the press-fit location for pipette tip **90**; and a smaller-diameter segment **43c** at the end of reagent tip head **43**.

Figure 7 shows a series of views of wash tip **41** and blow tip **42**. On the left of the figure is a sectional view of Z head **70**, depicting six vertical channels in this embodiment. One channel is generally provided for each of the fluid reservoirs of the apparatus, at a minimum one channel is provided for wash tip **41**, a further channel is for the blow tip **42**, and a further channel is provided for reagent tip head **43**. The reagent tip head **43** is not shown in the figure, for clarity of presentation of the remaining features. The hollow interior of blow tip **42** is not limited to the specific shape shown, although certain advantages are obtained for aspects of this interior shape and slit shape as described below. In general, the hollow interior **77** of the blow tip **42** can vary significantly in shape as long as sufficient access is provided for easy flow of gas into interior space **77** so that pressure differentials do not build up and cause differential exit of gas through exit slot **79**. Exit slot **79** is generally located on the bottom-most surface of blow tip **42** and is preferably a linear exit slit having a length substantially equal to the width of a standard microscope slide of the type selected for use in a particular operation. As there are different sizes of microscope slides, different wash and blow tips can be prepared for the dimensions of each such microscope slide or other specimen substrate as desired. As shown in the sectional view of Figure 5, access **78** between hollow interior space **77** and exit slit **79** is preferably provided so that gas leaving slit **79** exits at an angle to the vertical. By providing exit slit **79** at a slight angle and moving Z head **70** in a direction toward the obtuse angle formed between the gas wall and the microscope slide **130**, the removal of water or buffer from slide **130** is facilitated.

At the right side of Figure 8 is a view of a liquid distribution embodiment of the invention. There is portrayed a main vertically oriented hollow located at the center of the embodiment. An opening **76** shown at the top of the embodiment is used during the manufacture of the device, and will be blocked after the installation is completed. However, opening **76** can also be used as an inlet for an extra liquid supply. Wash tip **41** will be installed at the bottom end of the embodiment, and is not shown in the figure. On the side wall of the embodiment, there is a row of liquid solution inlet connectors. This array of parallel channels provides a path for the incoming liquid solution to be distributed to the main hollow portion, which can then flow down to wash tip **41**. The advantage of this design is that by using individual supply lines to deliver different solutions, interference and cross-contamination will be eliminated. In the present

embodiment, as depicted in Figure 10, at least six fluid reservoirs are provided for storage of the various solutions, which enable the apparatus of the invention to perform the wide range of analytic procedures. In this preferred embodiment, the reservoirs are provided for (1) solution to remove the embedding medium from mounted specimens ("de-waxing"), (2) immunohistochemistry buffer, (3) special stain buffer, (4) *in situ* hybridization buffer, (5) DI water, and (6) DEPC water.

The above-described solutions are channeled to wash tip **41**, via a fluid distributor **372** (Figures 7 and 8). One feature of this embodiment is the angle between the inlet cavities **73** and cavity body **76**. The fluids from distinct tubes are channeled into body **76** having a plurality of internal cavities to direct fluid flow to distinct output ports **373**. A major consideration for the orientation of the angle is to minimize fluid flow resistance and to prevent undesirable mixing of incompatible fluids. This design will also minimize cross contamination by providing distinct output ports for each input port. In addition, using individual valves for each liquid solution is a simple and effective way to control liquid solution supply individually; no back-flow will occur and cross-contamination will be minimized.

Several typical reagent dispensing patterns can be provided for dispensing reagents on a microscope slide **130**. Most slides will have a specimen region **132** and a second region **134** for including information on the slide. Because of the nature of the procedure for mounting specimens on slides, a specimen can be present at any location within region **132**. As previously discussed, the thin film of buffer that will be present when a reagent is dispensed onto the slide assists in ensuring that adequate reagent is applied to the specimen, regardless of where the specimen is located or the reagent is applied. However, in a preferred embodiment of the apparatus, the reagent is dispensed in a pattern, rather than a single location, so that the distance that a reagent must diffuse through the liquid film is reduced. Several typical reagent dispensing patterns are shown in Figure 23, although other patterns will prove equally useful.

Furthermore, 1/3 and 2/3 distribution patterns restrict dispensed reagent to a portion of region **132**, in order to conserve reagents if a specimen occupies only a limited portion of the region. This can prove valuable where the reagents are expensive, such as, e.g., nucleic acid probes. To take full advantage of this conservation strategy, it may also be desirable to restrict the dispensed reagent from spreading beyond the application area,

for example by the use of a fluid barrier. Special slides are available commercially which include pre-established fluid barriers, or the user can establish such barriers by creating a border around the specimen with a hydrophobic material, such as a marker (PAP) pen, also available commercially.

Figures 18 and 24 show views of a tray **190** intended to hold microscope slides. Tray **190** is formed into a series of individual wells **192** for microscope slides; the location of a single microscope slide **130** is shown by a dotted line in the right-most well of the plan view in Figure 18. The design of the well can be dimensioned to fit both 1" x 3" U.S. standard and 26 x 76mm European standard microscope slides, constructed of any desired material such as, e.g., glass or plastic. Although the well can be made to fit slides of any desired dimensions, so as to facilitate staining of entire sections, the slides will not necessarily be limited to single tissue sections. Slides with a plurality of distinct sections, such is in a tissue microarray, can also be utilized. Specimens comprising "DNA chips" and "protein chips" may also serve as the mounted specimens and be processed utilizing the apparatus of the present invention. Individual side walls **194** separate each well **192** from its adjacent wells to prevent accidental contact of liquid, such as might occur during a washing operation, and to prevent contamination between adjacent specimens. The open bottom of each well **192** allows buffer to drain through the bottom of tray **190** where it will be disposed of, typically in a drain bin **27** as shown in Figure 2. The open bottom of the well **192** will also permit the use of projections on the baseplate **22** to contact frosted portions of the slide **130** (typically region **134**) and raise the slide **130** above the tray **190**, so that the edges of the slide **130** in the region where reagent is applied are not in contact with the tray **190**, thus precluding a capillary wicking of reagent or other solution and possible cross-contamination of the specimen. The side walls **194**, retaining tabs **196** and bracing feet **197** closely and accurately retain microscope slides placed into the individual wells. A gap **198** is present at one end of well **192** to allow easy grasping of an individual microscope slide **130** between thumb and forefinger for insertion into and removal from tray **190**. The slide can be held using either frosted end **134** or specimen region end **132**. As discussed previously, the blow tip **42**, wash tip **41** and dispensing head sub-assembly **40** are integrated onto Z head **70** in one embodiment. Thus, when performing a blow operation, the blow tip head **42** is located very close to the surface of slide **130** which is set into the slide tray **190**, and the reagent tip head **43** at the same

height is positioned just in front of blow tip **42**. A row of head openings **199** at the front wall is designed to provide a space for reagent tip head **40** when blow tip **42** moves to the rear end of the slide **130**. Removable trays **190** are designed for ease of operation by allowing a user to place microscope slides **130** in a loading tray **190** outside the apparatus **10** in which the staining operations will occur. Another advantage of allowing trays **190** to be removed from the slide tray holder is to simplify the process of cleaning and other maintenance work. The tray **190** also will be adapted to fit precisely into other elements at the appropriate location on base plate **22**.

As shown in Figure 18, right tray support **206** and left tray support **207**, which are installed on the baseplate **22**, contain five slide trays **190** in a row, each of which is capable of containing 12 slides for a total of 60 microscope slides in a predetermined array. Figure 18 shows how a tray **190** is set onto tray supports **206/207**. On right tray support **206**, there are four sliding rods **195** installed horizontally, facing and against the outside wall on the right side of the tray **190**. The other end of sliding rod **195** (right side) is linked with a micro lever switch, which is in turn electrically connected to an in-position light **191**. The sliding rod **195** can freely move left or right. A detailed view is shown in Figure 25. When there is no tray on the tray support, the sliding rod **195** is at the left-most position, so that switch **193** is 'off' and the light **191** is likewise off. When a tray **190** is fully inserted into tray support **206** and at the right position, the right side wall of the tray **190** pushes sliding rod **195** to the right, pressing the micro lever switch to its 'on' position, then activating the light **191**. The light **191** then indicates that the tray **190** is now in the correct position and ready for staining. Another function for sliding rods **195** is to 'soft lock' the trays **190** and prevent them from moving when the apparatus **10** is in operation.

As shown in Figure 18, reagent vial holder **120**, located at the left side of slide tray support **207** is designed to hold up to 60 reagent vials and can accept reagent vials having a wide range of capacities, typically from 5mL to 25mL. Alternatively, a vial holder block (not shown) having appropriate dimensions could be utilized in place of the depicted vials. Such a block could include a bar-code and present a smaller vial, such as a screw-cap vial commonly used to contain small volumes of expensive reagents, in proper orientation for access by the reagent tip head **43** of dispensing head sub-assembly

40. Such a holder block can thus extend the useful volume range as low as is practical, typically as low as 0.1mL, a range commonly associated with nucleic acid reagents.

A detailed view of one embodiment of reagent vial holder **120** is shown in Figure 20. The total of 60 reagent vials are divided into four columns with 15 vials in each. The left column of reagent vial holder **120** contains reagent vials #1 to #15 starting from the upper left corner of reagent vial holder **120** (see Figure 20) and down to vial #15 in the same column in vial holder **120**. The remaining columns of reagent vial holder **120** holds reagent vials #16 to #60. The vial numbers can be engraved right at the position where the reagent vial will be located. These numbers correspond to the specific reagent vial numbers in the programming of the instrument. As shown in the figure, the spacing prongs **122** between two reagent vials (up and down, left and right) are designed to maintain certain spacing between adjacent vials, and accommodate shape/size distortions of the plastic reagent vials **110**. In this manner, the reagent vials will be secure in the proper positions. Handles **124** can be installed at both ends of reagent vial holder **120** to simplify manipulation. In a preferred embodiment, the reagent vial holder **120** is suspended by four corners. The corners and the resting position are maintained flat. This design allows for a stable configuration, which simplifies manufacture and control.

As also shown in Figure 18, the reagent vial **110** will also be configured so as to provide a region **112** for the bar-code label in a horizontal orientation on the upper surface of the vial. In this manner, the reagent vial information can be readily accessed at the same time as the location information. The use of the bar-code information from the various bar-codes will be covered in detail below.

One feature of the present apparatus is that the design which minimizes the risk of cross-contamination between specimens, reagents, and liquid solutions enables the use of the present apparatus for aspects of specimen preparation and specimen processing heretofore unavailable in an automated specimen staining device. For example, Figures 2 and 7 show various components used in the supply of gas and wash liquids to Z head **70**. A gas compressor **170** provides gas through flexible conduit **172** to movable arm **30**, Z head **70** and ultimately to blow tip **42**. A computer controlled switch controls gas pump on and off to control the gas supply. Buffer or other wash solutions are supplied by utilizing a pressure delivery system. Different liquid solution are pumped from individual reservoirs through individual supply tubing conduits **51**, movable arm **30**, Z head **70**,

dispensing head sub-assembly **40**, and finally to the common dispensing head **41**. The material used for supply conduit tubing must be selected individually for specific liquid solutions. For example, the tubing used for dealing solutions should be resistant to organic solvents and detergents, for example Viton-type tubing. The fluid source **53** can be any reservoir or plumbing system that supplies a fluid useful in any of the methods of the invention, and can vary within single apparatus, for example, the fluid source can be a carboy or other container filled with DI water or buffer and the fluid can be delivered by a pump. In a preferred embodiment, the fluid is preferably delivered by pressure differential such as can be supplied by a compressor **55** connected to an upper portion above the fluid level. The outlet of such a container is preferably located at or near the bottom of the container to facilitate complete evacuation of solution from the container. In one embodiment, the fluid course comprises a carboy pressurized to about five psi by a computer controlled compressor. A pressure gauge **57** (Figure 2) and a pressure relief valve (Figure 9) can be included so as to prevent excess pressure from building up in the system, which could lead to undesired fluid leaks and fluid delivery failure.

A plurality of fluid sources is employed in the preferred embodiment of the invention to provide different solutions for wash tip **41**. A plurality of different fluid sources can also be employed to allow rapid switching between different fluid sources that supply wash tip **41**. The fluid delivery control means can be any automated mechanism for opening and closing the conduit leading from the fluid source to wash tip **41**. In a preferred embodiment, the fluid delivery control means comprises an electrically operated valve **54** (Figure 9). The volume of liquid from the wash tip is controlled by the time of opening of the valve. A sensor **87**, positioned for example at the bottom of the reservoir, can be provided in the fluid reservoir to monitor the fluid level. A similar sensor **88** can also be provided in the waste carboy, positioned for example at the top of the carboy, to sense when the carboy is full and needs to be replaced.

Thus, the present apparatus enables the inclusion of a specimen preparation step in the automated protocols implemented by the control system. This is a feature previously unavailable in apparatus which process mounted specimens in a horizontal orientation. Representative of protocols useful in such specimen preparation protocols are the methods disclosed in PCT Publication WO 95/24498 and in U.S. Patent No. 5,578,452,

the entire contents of which is incorporated herein by this reference. In this manner, the use of volatile organic solvents in dealing operations can be minimized, and the apparatus can perform such operations without requiring special ventilation.

The laser bar-code scanner **410** will typically trace a selected trail when reading the bar-codes on both microscope slides **130** and reagent vials **110**. A batch processing method is generally used in bar-code reading in the present invention. Rather than reading the bar-codes and acting on the information individually, the laser scanner **410** scans the bar-codes by batch in order to increase processing speed. In a typical pattern, laser scanner **410** begins scanning at the left most slide **130** in tray #1 (top row). The scanner **410** on the movable arm **30** moves vertically down to the bottom row (tray #5), the scanner reading the bar-codes **402** on slide #1, #13, #25, #37 and #49. The information can be saved in laser scanner **410** as it scans, then transferred to the computer, e.g. via a RS232 serial port, using a protocol compatible for both. The most commonly used protocol in this regard is 7 bit data, 1 bit stop and even parity check. Movable arm **30** then moves one slide left, where laser scanner **410** is aiming at the microscope slides in the next column (#2, #14, #26, #38 and #50). For this pass, movable arm **30** moves from bottom to top, while laser scanner **410** reads the bar-codes **402** in reversed order. After reaching the top row of slide tray **190**, again the information can be transmitted to the computer. Thus, slide location information is automatically determined via software. If any bar-code is not properly read, or missing, the computer is capable of identifying which slide is "missing" and a menu on the computer screen informs the user to manually input the missing information or to re-run the scanning procedure. As soon as the microscope slide scanning is completed, movable arm **30** moves laser scanner **410** to the reagent vials **110** and the same steps can be used to read all desired information on the reagent vials **110**. One benefit of this batch processing strategy is that the apparatus can process trays of mounted specimens in a manner that completes the prescribed processing on a single tray **190**, and then signal the user to remove the tray and replace it with a fresh tray, without interrupting the processing of the remaining trays. In this manner, the apparatus can be utilized continuously with a minimum of required intervention by the user.

It will be apparent to one of ordinary skill that many of the specific elements shown in the figures and described above can be replaced by other elements that perform

the same function. For example, a single robotics arm can replace the X, Y, and Z tracks. Additionally, it will be understood that the specific tracks, motors and other individual parts can be replaced by other parts of equivalent function. In a preferred embodiment of the invention, the X, Y, and Z tracks are purchased as commercially available sliding tracks. The X-axis and Z-axis motions are supplied by a linear motion rail assembly and linear motion guide (Part Nos. RSR 15ZMUU + 700LM from THK, H/LCR and SEBS 9AUU-I-155 respectively from Specialty Motions, Inc.) and stepping motors (Part No. PK545-NAA for the X-axis and PK544-NAA for the Z-axis, both supplied by Automation Motor Controls). The Y-axis shaft system is a linear bearing rail assembly (Part No. RRPAC0134A also supplied by Pacific Bearing); power along the Y-axis is supplied by stepping motor (Part No. PK566-NBA). Electrical connections (as depicted in Figure 14), tubing, compressed gas distribution systems, liquid distribution systems from a central reservoir, and many other components such as holders for a standard pipette tip rack or for reagent containers are commercially available from a variety of suppliers. Thus, the apparatus of the invention can be prepared from readily available commercial parts assembled in the manner described, with a minimum number of specialized manufacturing techniques. Since the Z head **70** is not readily available, it will typically be manufactured for a particular apparatus as shown or in a similar manner to provide the features that are described in the specification above.

The composition of the components from which various parts are manufactured can vary widely, but components through which reagents pass or which contact potentially corrosive reagent or wash solutions are typically prepared from stainless steel or inert plastics to prevent corrosion. The Z head **70** with its integrated tips and other features is typically formed from a moldable plastic (such as a poly methyl methyl acrylate) and can be prepared by a molding process, a plastic-shaping process, or some combination thereof depending on the individual shape intended to be utilized. Parts that are subject to wear, such as the slide **38** and reagent tip head **43** of Z head **70** are typically prepared from a hard plastic or other material that will resist wear.

The present apparatus is typically operated under the control of a computer or other programmable control device. In the simplest applications, where only a single type of automated staining well be performed repeatedly, it is possible to provide either a hard-wired controller or a non-programmable electronic controller, such as a computer

operating under instructions from read-only memory. In preferred embodiments, however, a programmable controller or computer is used so that the operation of the apparatus can be varied. Software will generally be provided with the computer so that the user does not need to provide instructions for individual motions, but merely selects appropriate motions from a menu. In an 'open' format, for a typical operation, a user would be asked to select the location and volume of the reagents, the location of the specimens being treated, and the length of time for various steps such as incubation times; all other operations can be carried out by the pre-programmed instruction set in the memory of the computer, which will control actual movement of the movable arm to the appropriate locations and activation of the various gas and liquid control systems.

In a 'closed' format, bar-code technology can be used to supply instructions to the apparatus. The apparatus reads bar-codes associated with both the reagent vials **110** and the slides **130**; thereafter the computer is able to determine all parameters needed to carry out the most appropriate pre-programmed instruction set in the memory of the computer to control the apparatus in the processing procedures for mounted specimen staining. Compared with the 'open' format, less user input is required, thus reducing the opportunities for introduction of error.

The method that is used in the automated apparatus of the invention involves blowing excess reagent or buffer off the surface of the slide **130**. A preferred embodiment of the tip used in this blowing operation is shown in Figure 5, although other tips having slits for exit of gas to provide a wall of gas can also be used. By adjusting the gas pressure, height of the exit slit above the mounted specimen, and rate of movement of the slit, the extent to which liquid is removed from the specimen can be varied. The amount of liquid present in the thin film on the specimen substrate's upper surface is quite small, typically from 2 to 25 microliters, more generally from 3 to 20, and preferably from 5 to 10. The area to be covered is generally approximately 15mm^2, providing a typical volume per surface area of approximately 0.13 to 1.7 microliter/mm^2. However, it is difficult to determine the actual volume being used, since the operation of blowing liquid off the top surface of a substrate causes liquid to adhere to other portions of the substrate, making measurement of the remaining liquid difficult. Thus, the volume of liquid present on the upper surface of the substrate at the end of a blow operation is best determined empirically. The maximum permissible volume is determined by the

stain being used and its concentration at the reagent application stage, since these factors affect the final concentration of the stain or other reagent on the surface of the specimen. Historical procedures developed for specimen preparation are generally described in terms of a particular reagent concentration, incubation time, and temperature. Accordingly, it is desirable to provide a minimum volume of liquid on the specimen in order to avoid having to change the concentration of reagents from the standard used in the industry. By adhering to this guideline, it is possible to use commercially available, ready-prepared stain solutions as reagents.

On the other hand, too little liquid on a specimen can cause problems in reagent spreading, particularly due to evaporation. Since buffer is added to a substrate prior to addition of reagent and motion of the moveable arm to pick up pipette tips and reagents takes time, the buffer must remain on the substrate until reagent is added, which further may not occur until after the preparation of other specimens. Since it is more efficient to prepare multiple specimens at one time, rather than to require repeated movements of the movable arm and repeated pickup motions form the various heads, a typical minimum volume of buffer would be that amount which is sufficient to allow preparation of at least four specimens without requiring removal of a given tip.

The gas pressure, height of the head above the specimen and rate of motion of the head for control of the liquid film can all be selected by the user or by the manufacturer of the apparatus. Generally, the same gas pressure will be used at all times so as to remove this variable from consideration. Thus, only the height of the head and the rate of motion will typically be varied. The higher the head above the specimen, the less liquid will be removed. The faster the head is passed across the specimen for a given height, the less liquid will be removed.

For a standard 1 inch by 3 inch microscope slide surface area and a blow tip having the configuration shown in Figure 5, an gas pressure of 7psi (0.5cm/sec), a height of 0.07 inch (2mm) above the microscope slide surface, and a rate of motion of 3 inch/sec (7.5cm/sec) provide a preferred buffer film suitable for the staining of four slides at 25EC and a relative humidity of 60-80%, which is the typical humidity present inside a closed and operating apparatus of the invention.

The wash solutions used in the apparatus of the invention can vary significantly depending on the staining technique being used. A typical wash solution is an aqueous

solution of a surfactant and can contain other components present in typical specimen preparation of wash solutions, such as buffers.

In preferred embodiments, sufficient surfactant is present to provide a surface tension in a solution equivalent to that present in solutions containing water and the following surfactants at the concentrations listed. Typical surfactants used (with concentrations shown in parenthesis) are TWEEN™ 20 (0.02 to 2% v/v), BRIJ™35 (0.05 TO 3% v/v), and TRITON™ X-100 (0.01 to 1% v/v). Typical buffers used are phosphate buffered saline and TRIS-Cl (each at approximate pH 7.6). For conciseness of language, the specification and claims often refer to water as the wash fluid or the fluid being removed at a particular step. It will be apparent that this "water" can and generally is an aqueous solution of buffer and surfactant or of some staining reagent.

It will be apparent that the apparatus of the invention can be used in any staining technique that can be carried out manually and that there are no limitations placed on the invention by the staining technique.

The apparatus of the invention can contain a number of further components designed for ease of operation. For example, drain trays with exit conduits to waste reservoirs can be located either individually under components of the apparatus or a single drain tray and collection system can be provided for the entire interior space of the apparatus framework. In a typical apparatus, the framework is a form of a cabinet with an interior space in which all operations take place. A closeable access port (e.g., a door) can be provided to allow user access to maintain the various consumable components into the interior cabinet space. A transparent door can be provided to prevent accidental spraying of liquid (as during a blowing operation) into the room in which the apparatus is located, while allowing the user of the apparatus to visually verify proper operation. Other optional features that can be included on the apparatus include devices intended to ensure level operation, to protect against electric shock, to verify that an appropriate tip has been selected and properly placed on the tip head, or to optically scan specimens in a specimen substrate tray or other container so that a user is not required to enter information into the computer. Such information could be provided, for example, by a standard bar-code attached to an individual substrate or the component. Multiple reagent containers can be provided so that different staining operations can be carried out under the control of the bar-code system and the computer and its pre-programmed software.

It will be recognized that the apparatus of the present invention incorporates certain selected features from prior devices, particularly those which are the subject of U.S. Patent Nos. 5,439,649 and 5,948,359, the entire contents of which are each incorporated in their entirety by this reference.

All patents and patent applications cited in this specification are hereby incorporated by reference as if they had been specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent to those of ordinary skill in the art in light of the disclosure that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

REFERENCES

U.S. Patent Documents:

Patent No.	Issue Date	Inventors	Class
3,853,092	12/1974	Amos et al.	118/56
4,004,550	01/1977	White et al.	118/314
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5,180,606	01/1993	Stokes et al.	427/2
5,231,029	07/1993	Wootton et al.	435/289
5,439,649	08/1995	Tseung et al.	422/99

Other Publications:

"Ventana 320 Automated immunostaining system," Marketing brochure for Ventana Medical System, Inc., Tucson, AZ (date unknown).

"Jung Histostainer Ig Automated Immunostainer," Marketing brochure for Leica Instrument GmbH, (date unknown).

Claims:

1. An automated specimen processing apparatus for dispensing a reagent onto a mounted specimen comprising:

a supporting framework including at least one mounted specimen positioned thereon;

at least one reagent vial holder located on said framework and adapted for holding at least one reagent vial;

multifunction Z head comprising reagent dispensing means attached to said framework for dispensing a reagent onto the mounted specimen said reagent dispensing means comprising

at least one reagent tip head,

first control means operatively connected to said reagent dispensing means, said control means adapted to direct said reagent tip head to a position from which said reagent tip head can access the contents of said reagent vial, and

means for withdrawing fluid from said reagent vial and dispensing fluid onto the mounted specimen via said reagent tip head, and

second control means operatively connected to said reagent dispensing means, said control means adapted to cause said reagent tip head to detect the fluid level of the reagent contained in said reagent vial, and to perform at least one predetermined function based upon the detected fluid level.

2. The apparatus of claim 1, wherein said means for withdrawing fluid comprises means for alternatively supplying positive or negative gas pressure to said reagent tip head to withdraw fluid from said vial or dispense fluid onto said specimen.

3. The apparatus of claim 1, wherein said second control means comprises means for supplying positive or negative gas pressure to said reagent tip head and sensing means for detecting a change in the level of the gas pressure at said reagent tip head.

4. The apparatus of claim 2, wherein said means for supplying positive or negative gas pressure comprises a vacuum pump, a gas compressor, or a variable volume pump.

5. The apparatus of claim 2, wherein said means for supplying gas pressure is capable of withdrawing or dispensing a predetermined amount of gas through said reagent tip head, said predetermined amount being selectable by said control means, thereby providing precise withdrawing or dispensing of fluid into or from a disposable tip engaged with said reagent tip head.

6. The apparatus of claim 1, wherein said apparatus further comprises at least one tray releasably engaged with said framework and adapted to retain multiple mounted specimens and their associated substrates in a predetermined array.

7. The apparatus of claim 6, wherein at least one of said trays comprises a plurality of individual wells for each mounted specimen and the retention of the substrate of the mounted specimen in each of said wells is designed to minimize the loss of fluid distributed onto said mounted specimen and cross-contamination between said mounted specimens.

8. The apparatus of claim 6 wherein said tray is capable of engaging the mounted specimens from any orientation of the mounted specimen substrate.

9. The apparatus of claim 1 wherein said multi-function Z head further comprises a laser bar-code scanner adapted to read a bar-code.

10. The apparatus of claim 1 wherein the reagent vial comprises a reagent vial bar-code indicator to identify the location and description of each individual reagent vial.

11. The apparatus of claim 10 wherein at least one mounted specimen comprises a bar-code region including a bar-code indicator to identify the location and description of said mounted specimen on the tray.

12. The apparatus of claim 1, wherein at least said first control means comprises a programmable computer system and associated software for controlling and operating the functions of the apparatus.

13. The apparatus of claim 3, wherein said sensing means for detecting a change in the level of the gas pressure at said reagent tip comprises a pressure sensor, a static air pressure supply and a data acquisition feature.

14. The apparatus of claim 1 wherein said control means comprises a plurality of protocols for the control of the apparatus in processing said mounted specimens.

15. The apparatus of claim 14 wherein at least one of said protocols includes a plurality of formats for defining the process parameters of said protocol, and wherein at least one of said formats is a closed format which utilizes bar-code information to supply the information required for automatically processing mounted specimens.

16. The apparatus of claim 15 wherein at least one of said formats is an open format which permits a user of the apparatus to select at least one parameter to supply the information required for processing mounted specimens.

17. The apparatus of claim 14 wherein said plurality of protocols enables the apparatus to perform at least two processing protocols selected from the group of de-waxing protocols, denaturation protocols, immunohistochemical staining protocols, staining with special stains protocols, fluorescence *in situ* hybridization protocols and *in situ* hybridization protocols simultaneously during a single batch processing of mounted specimens.

18. The apparatus of claim 1 wherein said mounted specimen is mounted in at least one of the following formats: microscope slide, DNA chip and protein chip.

19. The apparatus of claim 18 wherein the mounted specimen is mounted on a microscope slide of any predetermined dimensions.

20. The apparatus of claim 1 wherein said multifunction Z head further comprises reagent tip sensing means for determining the presence of a disposable tip on said reagent tip head.

21. The apparatus of claim 20 wherein said reagent tip sensing means incorporates means for ejecting said disposable tip from said reagent tip head.

22. The apparatus of claim 21 wherein the operations of said multifunction Z head, said reagent tip head and the means for ejecting said disposable tip are controlled by a single motor.

23. The apparatus of claim 1 further comprising a plurality of fluid reservoirs for maintaining a supply of selected fluids to be used in the processing of said mounted specimen and fluid dispensing means for selectively dispensing any of said plurality of fluids onto said mounted specimen.

24. The apparatus of claim 23 wherein said fluid dispensing means further comprising a fluid distributor means for controlling the flow of the selected fluids from said fluid reservoirs while minimizing fluid flow resistance and fluid cross contamination.

25. The apparatus of claim 23 further comprising a means for detecting the fluid level in at least one of said plurality of fluid reservoirs.

26. The apparatus of claim 1 further comprising a gas dispensing means for selectively dispensing at least one gas onto said mounted specimen.

27. The apparatus of claim 26 further comprising at least one gas reservoir containing a supply of gas to be used in the processing of said mounted specimen.

28. The apparatus of claim 1 further comprising at least one fluid reservoir for receiving at least one fluid previously used in the processing of said mounted specimen and means for receiving said fluid from said mounted specimen.

29. The apparatus of claim 28 further comprising means for detecting the fluid level in at least one of said fluid reservoirs.

30. The apparatus of claim 1 wherein said means for detecting the fluid level of the reagent incorporates at least one of pressure sensing means, optical sensing means and electrical sensing means.

31. The apparatus of claim 1 wherein said supporting framework comprises a single piece support base means for locating and supporting the said mounted specimen, said reagent vial holder, and a supply of disposable tips in said apparatus.

32. The apparatus of claim 31 wherein the apparatus further comprises means for identifying and recording the relative positions of said mounted specimen, said reagent vial holder, said supply of disposable tips and said multifunction Z head in said apparatus.

33. The apparatus of claim 32 wherein said identifying means includes a calibration function based upon a single mechanical reference point located on said supporting framework.

34. The apparatus of claim 32 wherein said identifying means includes a calibration function based upon optical detector means and a single optical reference point located on said supporting framework.

35. The apparatus of claim 31 wherein the single piece support base means of the apparatus further comprises means for supporting said mounted specimen, said reagent vial holder, and said supply of disposable tips by suspension of racks or trays supporting these components, thereby insuring reliability and stability of the relative positions of these components and minimizing the effect of any variability due to manufacturing tolerances of the component support racks or trays.

36. The apparatus of claim 35 wherein said means for supporting said supply of disposable tips includes retention means to insure the stability of the relative position of said supply of tips during the operation of said apparatus.

FIGURE 1

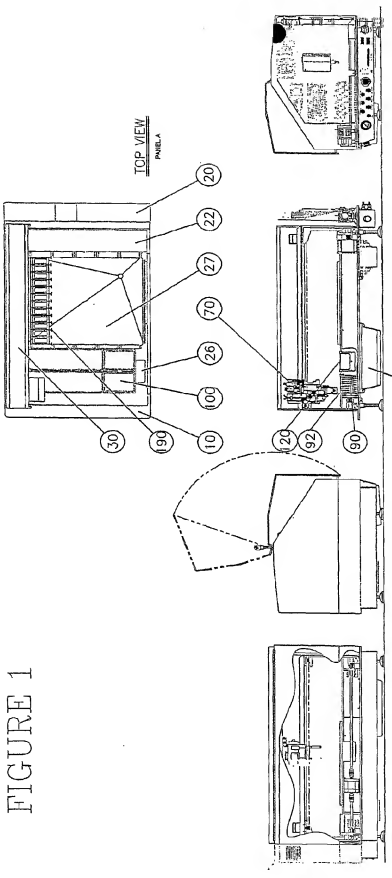

TOP VIEW
PANEL A
FRONT VIEW
PANEL D
RIGHT VIEW
PANEL E
LEFT VIEW
PANEL C
REAR VIEW
PANEL B
20
22
27
70
26
100
10
90
30
28
20
92
90

FIGURE 2

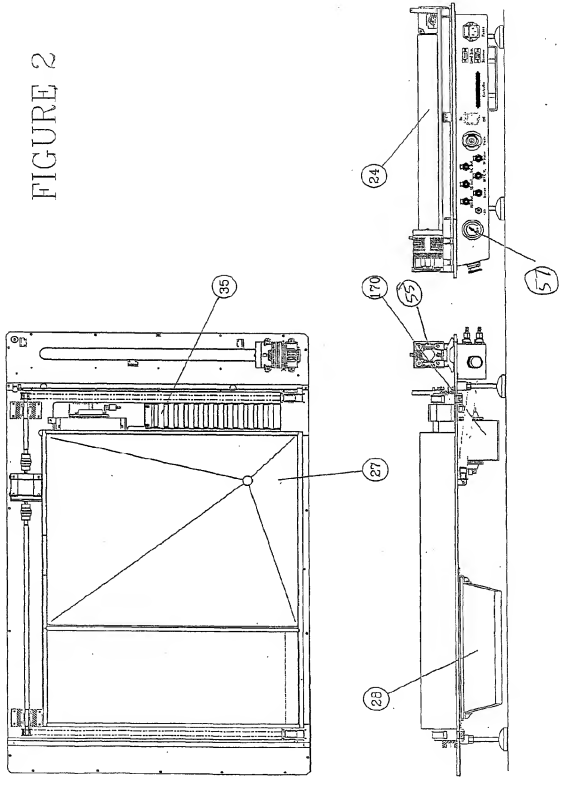
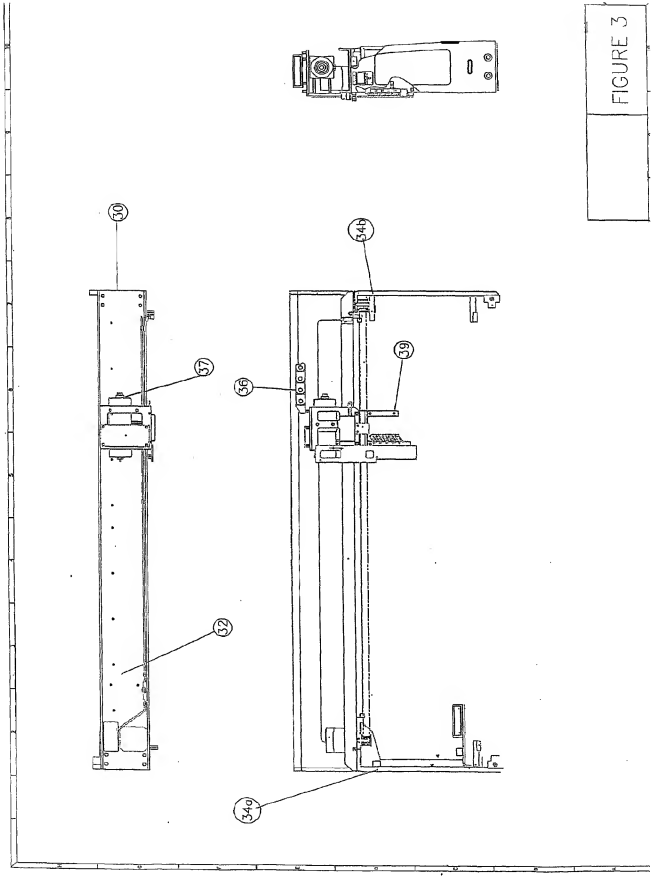


FIGURE 3



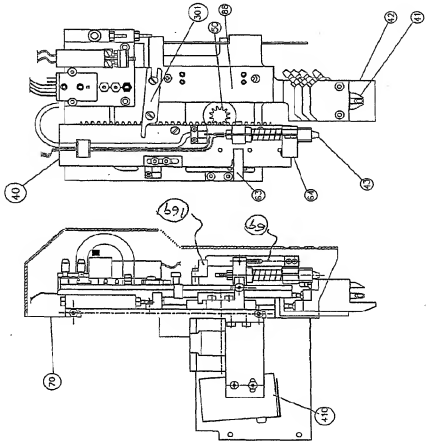


FIGURE 4

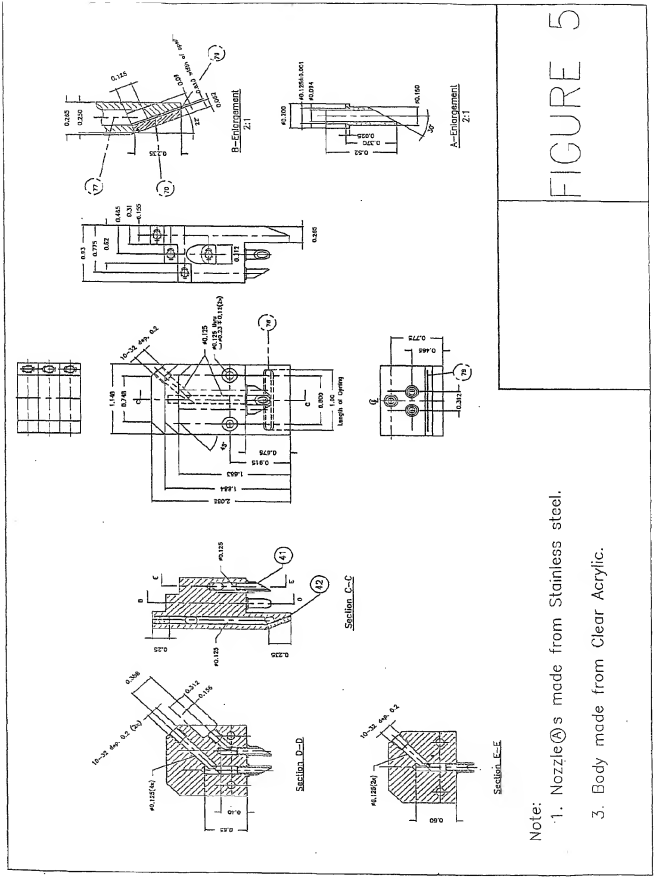
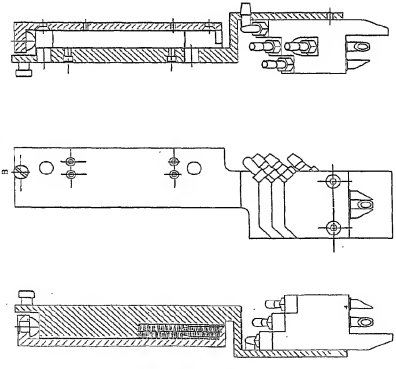

B-Enlargement 2:1
A-Enlargement 2:1
Section D-D
Section C-C
Section E-E
Note:
1. Nozzle(A)s made from Stainless steel.
3. Body made from Clear Acrylic.

FIGURE 5

FIGURE 6



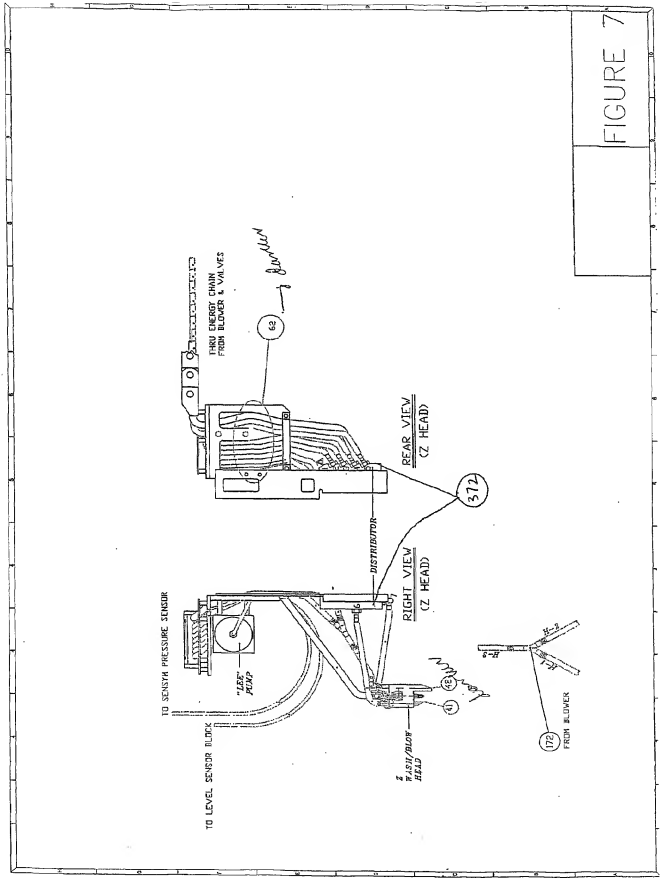


FIGURE 7
THRU ENERGY CHAIN FROM BLOWER & VALVES
REAR VIEW (2 HEAD)
RIGHT VIEW (2 HEAD)
DISTRIBUTOR
TO SENSYM PRESSURE SENSOR
TO LEVEL SENSOR BLOCK
"LEE" PUMP
FROM BLOWER

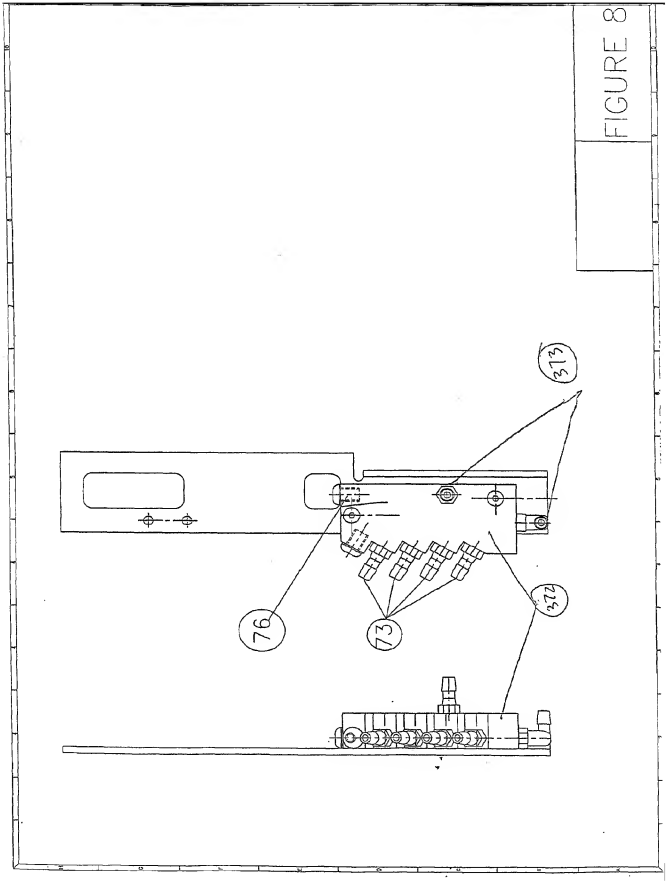


FIGURE 8
313
76
73
272

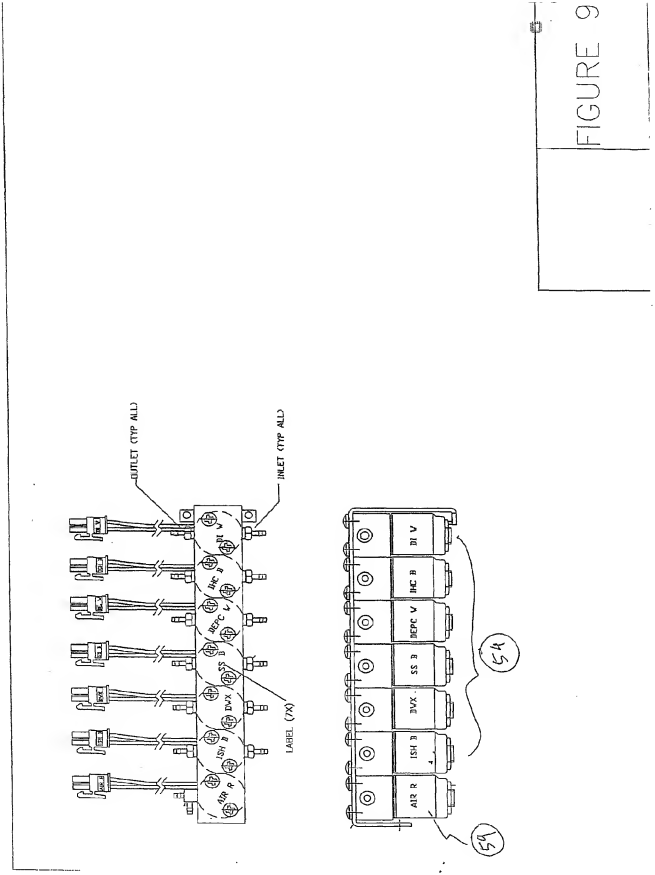

OUTLET (TYP ALL)
INLET (TYP ALL)
LABEL (7A)
AIR R
ISH B
DVX
SS B
DEPC V
DHC B
DI V
54
54A

FIGURE 9

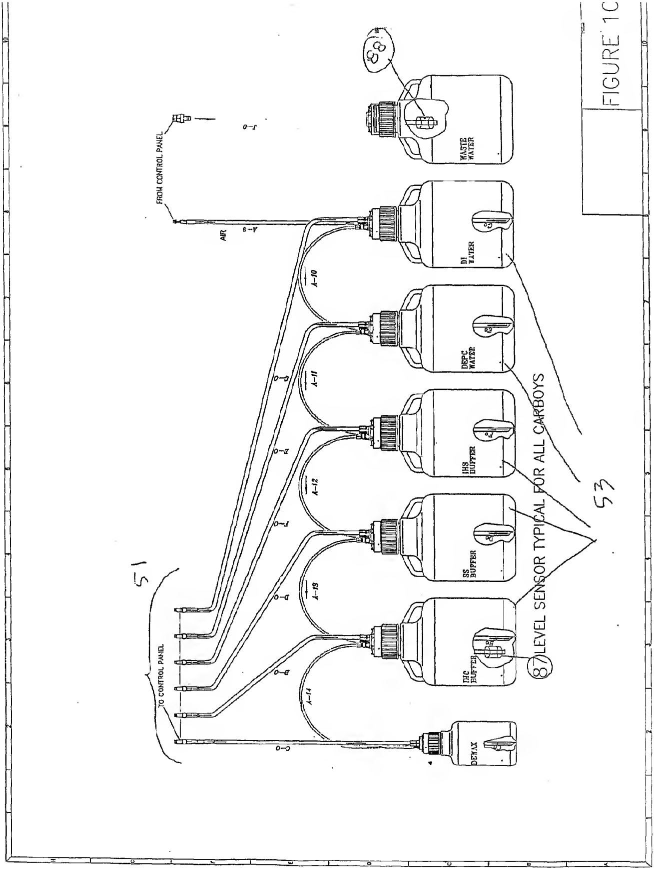
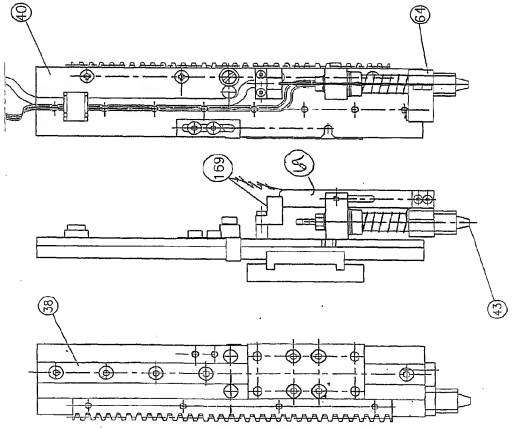

FROM CONTROL PANEL
AIR
WASTE WATER
88
DI WATER
DEPC WATER
IHS BUFFER
SS BUFFER
IHC BUFFER
DEWAX
TO CONTROL PANEL
51
LEVEL SENSOR TYPICAL FOR ALL CARBOYS
53
87

FIGURE 1C

FIGURE 11



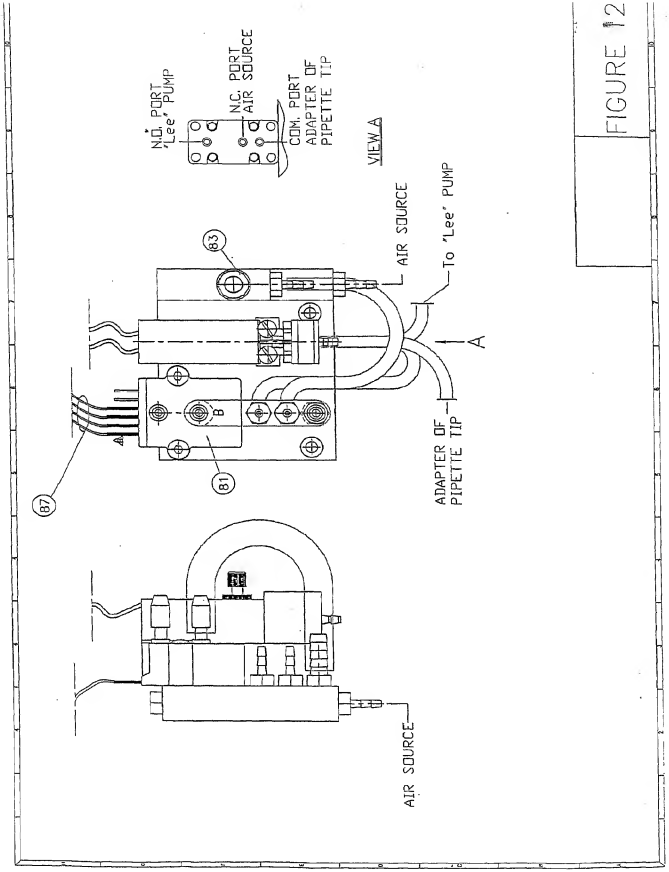

N.O. PORT
'Lee' PUMP
N.C. PORT
AIR SOURCE
COM. PORT
ADAPTER OF
PIPETTE TIP
VIEW A
AIR SOURCE
To 'Lee' PUMP
A
ADAPTER OF
PIPETTE TIP
AIR SOURCE
B7
B1
B3
A
B

FIGURE 12

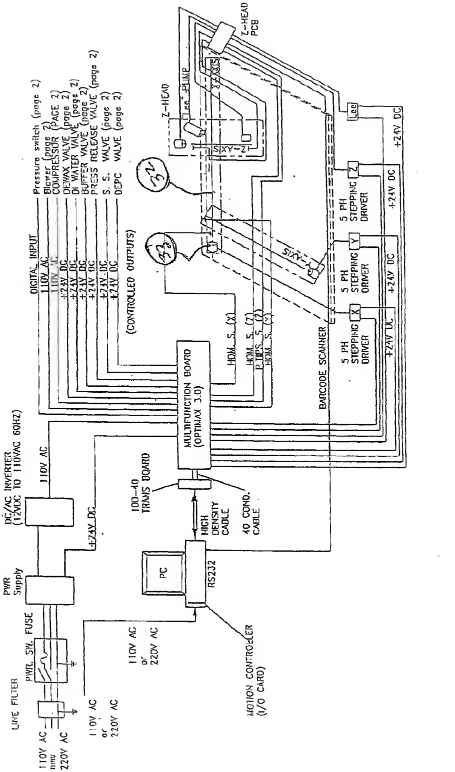

110V AC
thru
220V AC
LINE FILTER
PWR. SW.
FUSE
PWR Supply
DC/AC INVERTER (12VDC TO 110VAC 60HZ)
110V AC
+24V DC
110V AC
or
220V AC
PC
RS232
MOTION CONTROLLER (I/O CARD)
HIGH DENSITY CABLE
40 COND. CABLE
100-40 TRANS BOARD
MULTIFUNCTION BOARD (OPTIMAX 3.0)
DIGITAL INPUT
Pressure switch (page 2)
Blower (page 2)
COMPRESSOR (PAGE 2)
DEWAX VALVE (page 2)
DI WATER VALVE (page 2)
BUFFER VALVE (page 2)
PRESS RELEASE VALVE (page 2)
S. S. VALVE (page 2)
DEPC VALVE (page 2)
(CONTROLLED OUTPUTS)
HOM_S_(X)
HOM_S_(Z)
P_TIPS_S_(Z)
HOM_S_(Y)
BARCODE SCANNER
5 PH STEPPING DRIVER
X
Y
Z
+24V DC
Z-HEAD
Z-HEAD PCB
Y-AXIS
32
33

FIGURE 13

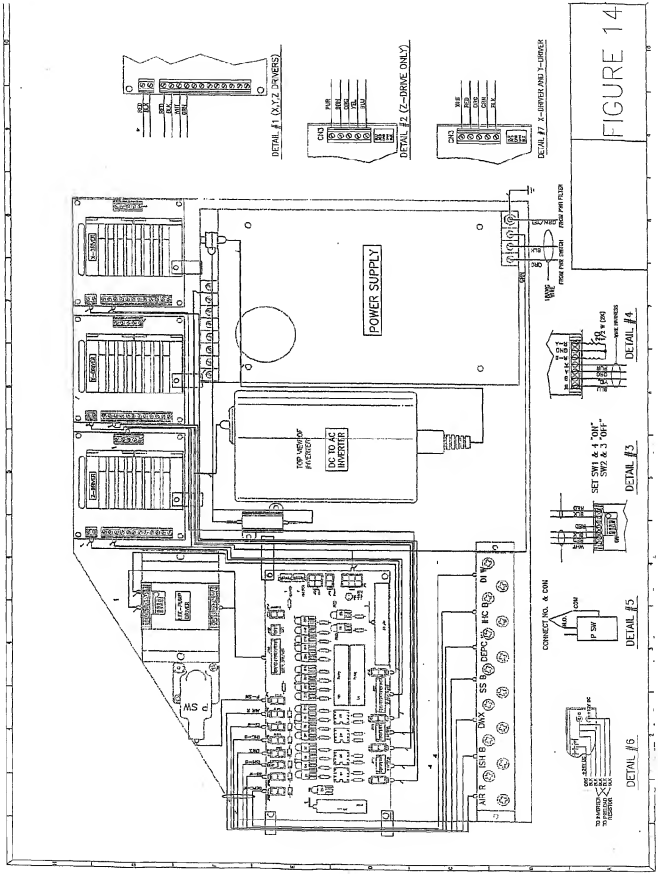


FIGURE 14
POWER SUPPLY
DETAIL #1 (X,Y,Z DRIVERS)
DETAIL #2 (Z-DRIVE ONLY)
DETAIL #3
DETAIL #4
DETAIL #5
DETAIL #6

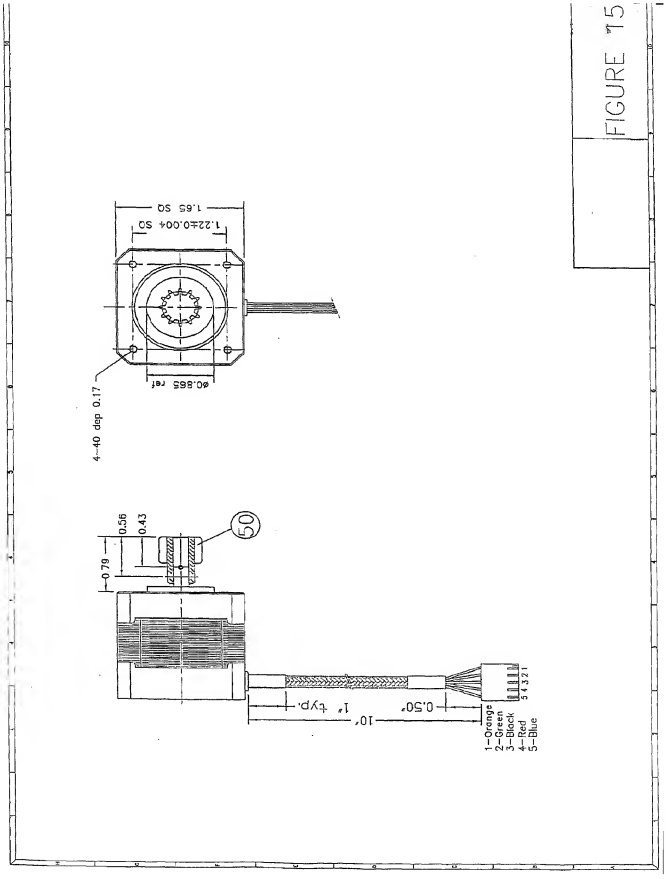

1.65 SQ
1.22±0.004 SQ
ø0.865 ref
4-40 dep 0.17
0.56
0.43
0.79
50
10"
1" typ.
0.50"
1-Orange
2-Green
3-Black
4-Red
5-Blue
5 4 3 2 1

FIGURE 15

ITEM #	PART #	DWG #	QTY	DESCRIPTION	REMARK
1	6520-20315	950-6520-20315	1	ASSY, Y-SHAFT(S)	
2	4250-04005		4	MOUNT, SHOCK	
3	6520-30230	950-6520-30230	1	ASSY Y MOTOR	
4	6520-20267	950-6520-20267	1	PAD, Y-MOTOR MOUNT	
5	4730-01240		2	ELEMENT COUPLING	Mc. #6410K35
6	6520-20316	950-6520-20316	1	ASSY, Y-SHAFT(L)	
7	6520-20268	950-6520-20268	2	ASSY Y-BEARING MOUNT	

FIGURE 16

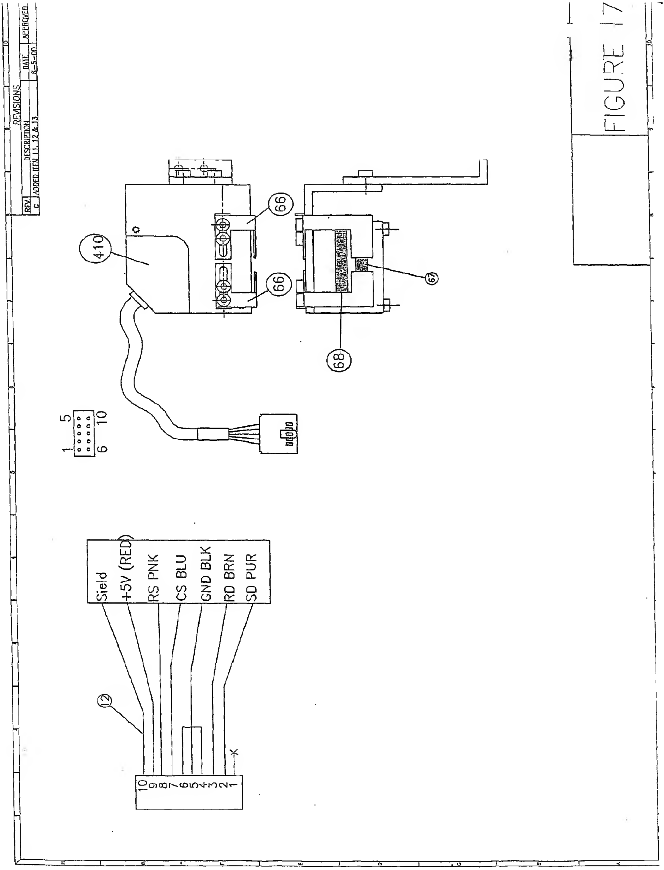

REVISIONS
REV
DESCRIPTION
DATE
APPROVED
G
ADDED ITEM 11, 12 & 13
6-5-00
410
66
66
67
68
1 5
6 10
Sield
+5V (RED)
RS PNK
CS BLU
GND BLK
RD BRN
SD PUR
12
10 9 8 7 6 5 4 3 2 1
X

FIGURE 17

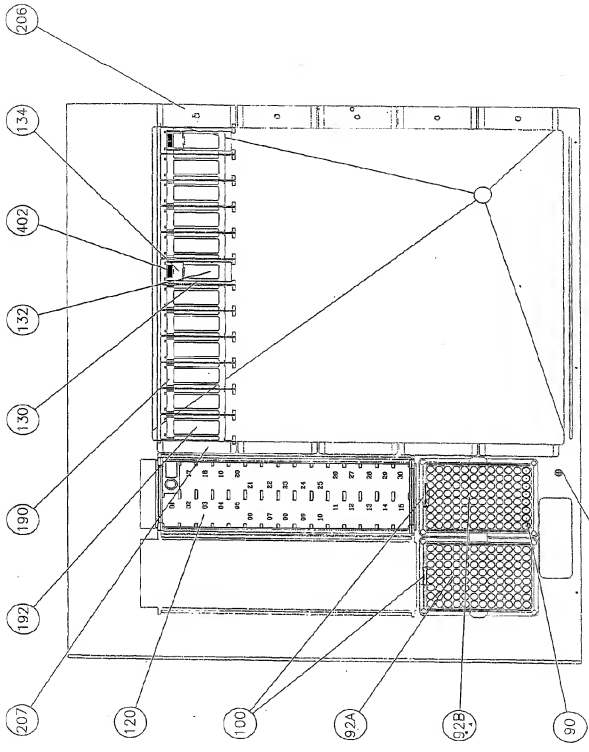


FIGURE 18

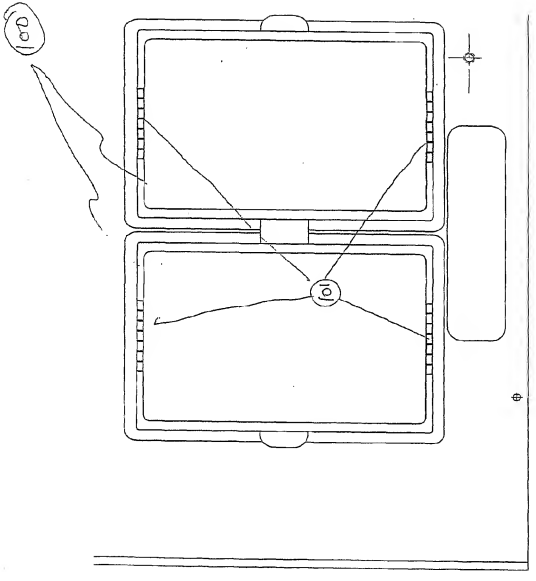
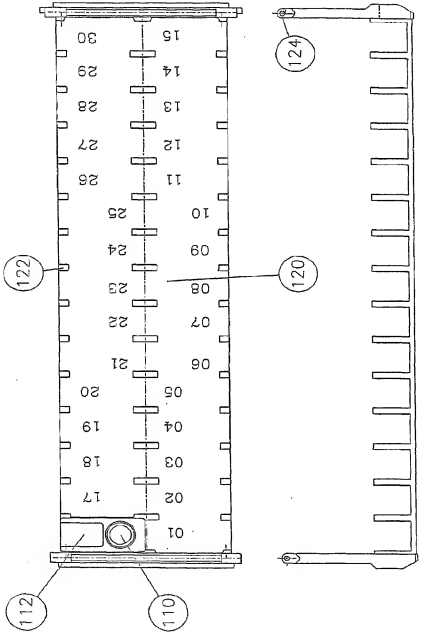


FIGURE 19


01
02
03
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120
122
124
110
112

FIGURE 20

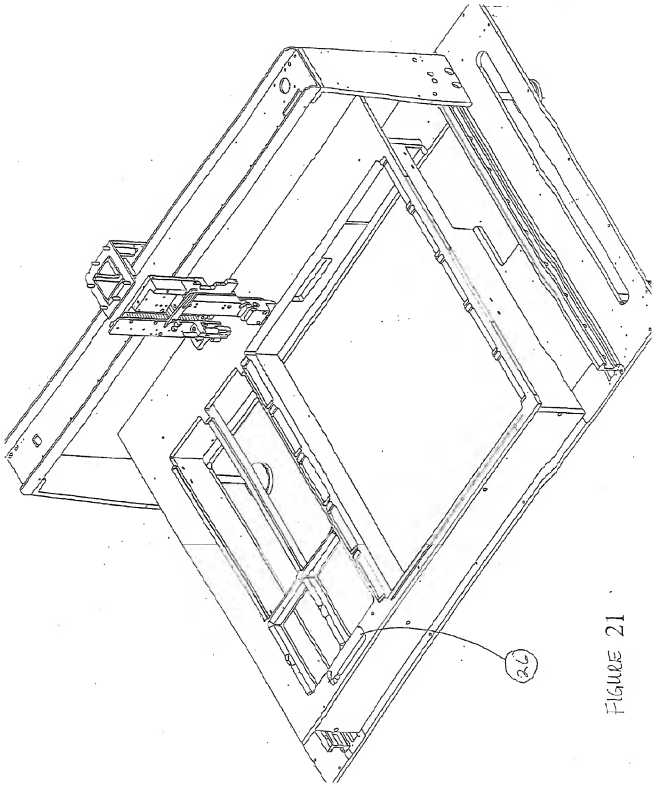

FIGURE 21

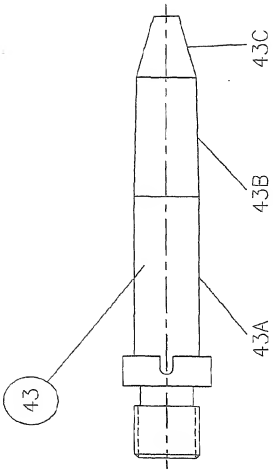

FIGURE 22

XAMPLES OF TYPICAL REAGENT DISPENSE PATTERNS

FIGURE 23

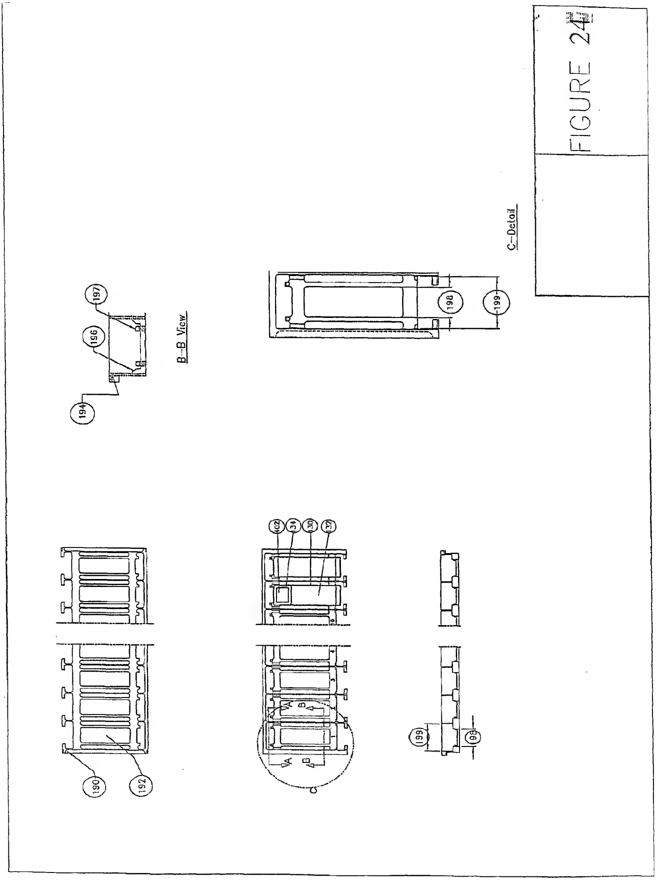

FIGURE 24

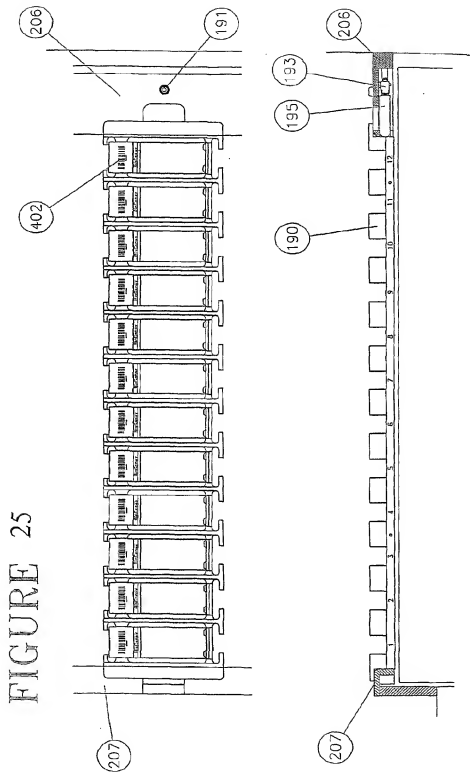

FIGURE 25

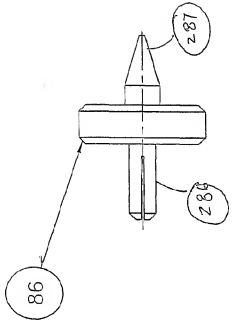

FIGURE 26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/08386

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : B01L 3/02; G01N 21/00, 31/00, 33/00; B32B 27/04, 27/12, 5/02
US CL : 422/100, 65, 67

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/100, 65, 67

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST DATABASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,948,359 A (KARLA et al.) 07 SEPTEMBER 1999, entire document	1-36
X	US 5,439,649 A (Tseung et al.) 08 August 1995, entire document	1-36
X	US 5,104,621 A (PFOST et al) 14 April 1992, abstract; column 9 lines 17-30; column 10, line 50-column 11, line 28; column 12 line 56-column 13, line 50; Fig 8; column 20, line 51-column 22, line 62	1-8, 12-14, 16-17, 20-25, 28, 30-32, 35-36
Y	US 5,306,510 A (MELTZER) 26 April 1994, abstract; column 5 line 50-column 6, line 69; column 8, line 42-62; column 9, lines 15-50	1-8, 12, 14, 16, 20-24, 26-28, 31-32, 35-36

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 22 MAY 2001	Date of mailing of the international search report 15 JUN 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BRIAN GORDON Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998) ★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/08386

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,906,795 A (NAKASHIMA et al.) 25 May 1999, abstract, column 1, lines 17-29; column 4 lines 18-28; column 8 line 46-column 9 line 6; column 14 line 40-column 16 line 49;	1-8, 12-13, 17, 20-22, 28-31, 35-36
Y	US 5,494,828 (LEOPANDO) 27 February 1996, entire document	2-3, 18-19
A	US 5,460,783 A (HAUTEA et al.) 24 October 1995, abstract; column 3 lines 14-25, column 4 lines 2-66	1-4, 27
A	US 5,356,595 A (KANAMORI et al.) 18 October 1994, column 3 lines 12-68; column 4, lines 8-34; column 6 lines 30-52; column 7 lines 19-27	1, 10-12, 17-19
Y	US 5,897,837 A (MIZUNO) 27 April 1999, abstract; column 1 line 50-column 2 lines 50; column 3 lines 16-21, column 4, lines 27-37; column 6, lines 19-50	1-8, 12, 14, 17, 23, 26
Y, P	US 6,105,636 A (SCATIZZI et al.) 22 August 2000, entire document	1-3, 5-8, 12, 24, 28
Y, P	US 6,182,719 B1 (YAHIRO) 06 February 2001, abstract; column 1, line 52-column 2, line 14; column 2 line 64-column 3, line 17	1-3, 7, 20-21, 31, 36
Y, E	US 6,212,949 B1 (INDER et al.) 10 April 2001, abstract; column 2 lines 31-column 3 line 31; column 4 lines 19-48	1-8, 12, 22, 25, 29
Y, E	US 6,234,033 B1 (EIPEL) 22 May 2001; entire document	1-8, 13, 1819, 23, 27